



## KIDNEY FUNCTION IN PNEUMONIA

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### INTRODUCTION

Studies of the changes which occur in kidney function during the course of lobar pneumonia have been made by several investigators. The results which they have obtained show little uniformity, and the conclusions arrived at are quite at variance. Tileston and Comfort (1) studied 14 cases of lobar pneumonia and found an elevation in the blood urea during the febrile stage. They made 8 phenolsulphonphthalein tests, and found a diminution in excretion in 3 instances. Schwartz and McGill (2) in a series of 20 cases, obtained very similar findings. Longcope and Peters (3) studied 4 cases of pneumonia incidental to their study of renal function in serum disease. The phenolsulphonphthalein excretion was below normal in only one of their 6 determinations, and the blood urea was normal in all instances. They compared the urea in the urine with the urea in the blood by means of McLean's index, and found normal values in all their determinations. Bookman (4), on the other hand, found a diminished phthalein output in 5 of his 6 cases of pneumonia, and considered that without other signs of nephritis, most cases showed distinctly impaired function. The blood urea showed no striking change in his series. Lewis (5) investigated 8 cases of lobar pneumonia in his study of the clinical value of Ambard's "coefficient of urea excretion." He found a well marked increase in the phenolsulphonphthalein excretion, the average for 2 hours being 70.5 per cent, compared with 60.2 per cent in his series of 28 normal cases. The values given for the blood urea nitrogen were all normal, but the output of urea in the urine was higher than one would expect in a normal individual. Lewis points out that "any kidney capable of raising the concentration of the urinary urea to a level 80 times that in the

blood can scarcely be called a hyperpermeable organ," but that "the increased rate of output indicates an increased functional ability of the kidney."

Frothingham (6) failed to find a general increase in phenolsulphonphthalein excretion. Of 15 determinations done on 10 cases, only one (which was done during the febrile stage) showed an increased output (83 per cent), while decreased outputs were obtained twice. Five of the cases showed a McLean index higher than normal during the febrile stage, which returned to normal limits during convalescence in the four instances in which the test was repeated. The index was subnormal in 3 determinations, once during the disease, and twice after the patients were afebrile. This author concludes that since the increase in the index was not associated with an increase in phenolsulphonphthalein a hyperactivity of the kidney does not occur during fever. He also considered that the tests failed to show consistent evidence of impaired renal function during the course of the disease.

Of the six authors, therefore, three emphasize the finding of impairment of kidney function, two emphasize the absence of impairment, and one emphasizes the occurrence of hyperfunction during the course of lobar pneumonia. It seemed to us, therefore, that a series of observations, repeated at intervals during the course of the disease, might throw some light on the cause of such discordant results. The urea concentration index, recently described by Van Slyke, Linder, Hiller, Leiter and McIntosh (8), by means of which the concentrating power of the kidney for urea under standard conditions is estimated, seemed to be a convenient method for the study. It also seemed to us that such a study would be necessary before this test could be used as a diagnostic measure in suspected cases of nephritis, in which a febrile disease, such as pneumonia, was present, at the time of the test, or shortly antecedent to it.

#### METHODS

In the material presented, the phenolsulphonphthalein was given intravenously, and the specimens collected at the end of one hour, and 2 hours, when this was possible. This object was not always achieved on account of the inability of the patients to void at the

proper time. Such results were discarded. It seemed to us that in view of the frequency of difficulty in voiding, phenolsulphonphthalein tests are not wholly reliable in such cases, unless catheterization is carried out. The urea concentration index is open to the same objection, but in this instance the error can be minimized by lengthening the period of urine collection. In this study, all urine collections which were open to suspicion of being incomplete were discarded. The patients were given 150 cc of water fifteen minutes before the first voiding, and urine collections were made over a period of 2 hours as a standard procedure. The urine and blood urea was determined by the urease method of Van Slyke and Cullen (7) except in a few urine urea determinations, which were made by a gasometric method which will shortly be described by Van Slyke. The significance of the urea concentration index may be described by stating that it represents the number of times the kidneys concentrate urea in excreting it from the blood into the urine, when the urine volume output is at the average normal rate of 1 cc per minute, or 1 cc per hour per kilo of body weight. Values of 35 to 80 for the index are regarded as being normal, and 55 per cent as the lower limit of normal for phenolsulphonphthalein determination.

#### RESULTS

In all, 13 cases were studied. The results are summarized in table 1. The most characteristic finding is to be noted in cases 1, 2, and 3. These show a normal index early in the disease, followed by a marked increase, which usually occurred before the crisis, and lasted in one case as long as seven days after it. The index subsequently returned to normal. In cases 4, 5, and 6 the same supra-normal indices were observed during the febrile period, and in each case, a return to normal occurred after the crisis. The phenolsulphonphthalein return showed the same supra normal phase, and a good parallelism can be observed between it and the index in cases 1, 2, 3, and 4.

None of the other cases showed definitely supra-normal indices. Nevertheless, the same tendency to hyperfunction can be observed in several of them. Case 9 showed a series of three indices between the fourth and the tenth day, which were probably considerably above his individual normal level. This case, as well as cases 8 and 12, also showed an increased output of phenolsulphonphthalein.



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## CONTENTS

NUMBER 1, OCTOBER, 1926

The Effect of Digitalis on the Cardiac Output of Dogs and Its Bearing on the Action of the Drug in Heart Disease. TINSLEY RANDOLPH HAR RISON AND BERNARD W LEONARD	1
Studies in Serum Electrolytes I Concentration of Electrolytes and Non-electrolytes in the Serum During Lobar Pneumonia F WILLIAM SUNDERMAN, J H AUSTIN AND J G CAMAC	37
An Experimental Study of Chronic Aortic Regurgitation in Dogs H C BAZETT AND JANE SANDS	65
The Significance of Electrocardiograms of Low Voltage. HOWARD B SPRAGUE AND PAUL D WHITE	109
Kidney Function in Pneumonia JOHN F MCINTOSH AND HOBART A REIMANN	123
An Experimental Study of the Synovial Fluid of Patients With Arthritis and Syphilis. ALAN M CHESNEY, JAROLD E KEMP, AND FREDERICK H. BAETJER	131
A Study of the Action of Ammonium Chlorid and Organic Mercury Compounds. NORMAN M KEITH AND MARY WHELAN	149

NUMBER 2, DECEMBER, 1926

Benign Glycosuria Due to Disturbances in the Blood Sugar Regulating Mechanism. KNUD FABER	203
Studies on Red Cell Diameter I In Health and in Pernicious Anemia J R BELL, F K THOMAS, AND J H MEANS	229
The Effect of Increased Heart Rate Due to the Injection of Atropine on the Oxygen Saturation of the Arterial and Venous Blood of Patients with Heart Disease HAROLD J STEWART	241
Influence of Previous Salt Regime on Excretions of Chlorine, Sodium, and Potassium During the Chloride Concentration Test of De Wesselow LOUIS LETTER	253
The Surface Tension of the Blood Serum in Nephritis LOUIS LETTER	267
The Physiological Response of the Circulatory System to Experimental Alterations III The Effect of Aortic and Pulmonic Stenoses. EMILE HOLMAN AND CLAUDE S BECK	283
The Association of Generalized Arteriolar Sclerosis with High Blood Pressure and Cardiac Hypertrophy in Chronic Nephritis. ARNOLD BRANCH AND GEOFFRY C. LINDER	299

The Effect of Breathing Oxygen-Enriched Air during Exercise upon Pulmonary Ventilation and upon the Lactic Acid Content of Blood and Urine A W HEWLETT, G D BARNETT AND J K LEWIS	317
Calorimetric Studies of the Extremities I Theory and Practice of Methods Applicable to Such Investigations CHARLES SHEARD	327
Calorimetric Studies of the Extremities II Experimental Apparatus and Procedures ROY KEGERREIS	357
Calorimetric Studies of the Extremities III Clinical Data on Normal and Pathologic Subjects with Localized Vascular Disease GEORGE E BROWN	369
Studies in Scarlet Fever I The Amount of Scarlatinal Toxin in the Blood of Patients with Scarlet Fever JAMES D TRASK	391
Studies in Scarlet Fever II The Relation of the Specific Toxemia of Scarlet Fever to the Course of the Disease FRANCIS G BLAKE AND JAMES D TRASK	397
Studies in Scarlet Fever III Infections with Streptococcus Scarlatinae in Persons with Scarlatinal Antitoxic Immunity EDITH E NICHOLLS	411
Studies in Scarlet Fever IV Post-scarlatinal Immunity in Patients Treated with Antitoxin J A V DAVIES	423
The Effect of Tachycardia on the Blood Flow in Dogs I The Effect of Rapid Irregular Rhythms as Seen in Auricular Fibrillation HAROLD J STEWART, J HAMILTON CRAWFORD AND A B HASTINGS	435
The Effect of Tachycardia on the Blood Flow in Dogs II The Effect of Rapid Regular Rhythm HAROLD J STEWART AND J HAMILTON CRAWFORD	449

NUMBER 3, FEBRUARY, 1927

Epidemic Prevalence in the Light of Experimental Findings L T WEBSTER	465
A Technique for Measuring X-Ray Photographs of the Cardiac Areas of Dogs HAROLD J STEWART	475
The Effect of Regular and Irregular Tachycardias on the Size of the Heart HAROLD J STEWART AND J HAMILTON CRAWFORD	483
The Plasma Proteins in Relation to Blood Hydration III The Plasma Proteins in Malnutrition JOHN P PETERS, A MAURICE WAKEMAN AND ANNA J EISENMAN	491
Total Acid-Base Equilibrium of Plasma in Health and Disease VIII Bicarbonate and Chloride in the Serum of Patients with Heart Failure JOHN P PETERS, HAROLD A BULGER AND ANNA J EISENMAN	497
Total Acid-Base Equilibrium of Plasma in Health and Disease IX High Serum Bicarbonate in Heart Failure Asphyctic Anoxemia JOHN P PETERS, HAROLD A BULGER AND ANNA J EISENMAN	511

## CONTENTS

v

Digitalis and Diuretics in Heart Failure with Regular Rhythm, with Especial Reference to the Importance of Etiologic Classification of Heart Disease H M Marvin	521
Studies on Red Cell Diameter II In Pernicious Anemia, Before and During Marked Remission and in Myelogenous Leukemia DONALD N MEDEARIS AND GEORGE R MINOT	541
The Mechanism of the Action of the Hydrogen Ion Upon the Cardiac Rhythm E COWLES ANDRUS AND EDWARD P CARTER	555
Epinephrin Reactions in Obesity CLEMENT I. KRANTZ AND JAMES H MEANS	565
Edema. II The Effectiveness of Ultrafiltration for Quantitatively Determining the "Free Water" Content of Blood Plasma, and for Estimating Physical Chemical Changes of the Plasma Proteins in Edema. MARCARETE META KUNDE	577
Dependence of the Form of the Electrocardiogram Upon the Site of Mechanical Stimulation of the Human Ventricles B S OPPENHEIMER AND HAROLD J STEWART	593
Variations in Blood Flow with Changes in Position in Normal and Pathologic Subjects JOHN S LAWRENCE, LEWIS M HURXTHAL AND A V BOCK	613
A Comparative Study of the Rotatory and Reducing Properties of Ultrafiltrates from Blood Plasma JOHN R. PAUL	631
Index to Volume III	653







# THE EFFECT OF DIGITALIS ON THE CARDIAC OUT-PUT OF DOGS AND ITS BEARING ON THE ACTION OF THE DRUG IN HEART DISEASE

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## INTRODUCTION

The mode of action of digitalis has been a subject of discussion and experimentation since Witherings' classical observations which introduced the drug into medicine. In spite of extensive investigation, the essential problem, the effect of the drug on the out-put of the heart of patients suffering from cardiac insufficiency, remains unsolved. Our knowledge along this line has been summarized by Robinson (1) as follows:

"The relation of the effect of digitalis on ventricular contractions to its beneficial influence in heart disease has been discussed but this problem has not as yet been definitely solved. In spite of the uncertainty which actually exists digitalis has been generally considered for many years as a so-called 'heart tonic' and its beneficial effect has been considered as mainly due to an increased out put of the heart by its action on the muscle itself."

The literature on digitalis has been carefully reviewed by Robinson and, more recently, by Cushny (2). In this paper only those studies which bear directly on the effect of the drug on cardiac out put will be mentioned.

Gottlieb and Magnus (3) and Magnus and Sowton (4) found that the work per beat of the perfused mammalian heart is increased by digitalis, the work per minute being either increased or decreased according to the degree of slowing. Cushny (5) using the cardiometer found an increase of 20 to 30 per cent in minute out put in the intact heart, while in the denervated or atropinized heart the effect was

variable, there being usually no significant change in out-put (6) Schmoll (7) holds that digitalis acts by affecting the tonicity rather than the contractility of the cardiac muscle, while others, notably Gottheib (8), Schmiedeberg (9) and Cushny (10) believe that the essential action lies in augmented contractility Cohn (11) points out that these discrepancies in results may be ascribed to variations in dosage and method, and emphasizes the fact that experimental doses have usually been in excess of therapeutic doses In 1915 Cohn stated that an increase in the out-put of the heart resulting from the action of digitalis in therapeutic doses had not been demonstrated either clinically or experimentally In 1920 Cohn and Levy (12) studied the effect of intravenous injections of digitalis and g-strophanthin in dogs and cats, by means of volume curves The results were somewhat variable, the output being usually but not always increased in dogs, and unchanged or diminished in most of the cats Strong and Gordon (13) showed that strophanthin given intravenously decreases the size of the heart of normal rabbits and of rabbits with experimental myocarditis Recently Levy (14) has demonstrated a decrease in the size of the heart as determined by the x-ray after digitalization in pneumonia, while Cohn and Stewart (15) have demonstrated an increased excursion of the ventricles in man as judged by "moving" orthodiagrams Vagt (16) calculated the cardiac out-put after digitalis by studying the arm volume and the blood pressure He concluded that the drug caused a slight increase in the stroke out-put and a slight decrease in pulse rate Kaufman (17) using another indirect method concluded that the out-put per beat was increased by 10 per cent and the out-put per minute decreased about 10 per cent All the above methods except the cardiometric are indirect and subject to many errors

The only direct measurements of the effect of digitalis on the cardiac out-put of man are those of Eppinger, von Papp and Schwarz (18) They used a modification of the Plesch method and during an extensive study of "cardiac asthma" they record two observations in which the effect of digitalis on the out-put of the heart in patients with heart disease was determined In one instance they found a definite diminution in the cardiac out-put per minute after the patients had received full therapeutic doses of the drug In the other case diminution

occurred, but its relation to the action of the drug is uncertain. These observations are the only direct measurements, so far as we are aware of the effect of digitalis on the out-put of the heart.

The effect of digitalis on the regularly beating heart of patients with cardiac insufficiency has been much discussed. The beneficial influence of the drug when auricular fibrillation is not present and when the cardiac rate is not definitely altered is naturally referred to its effect on the heart muscle. Under these circumstances it has been generally inferred that digitalis improves the efficiency of the heart by increasing its out put, and that this effect results from the direct action of the drug on the heart muscle. However there is not agreement that digitalis is usually beneficial under these conditions.

The foregoing review may be briefly summarized as follows:

1 A general impression prevails among clinicians that digitalis increases the out put of the heart.

2 The majority of pharmacologists support this view, their evidence being based on experiments with the perfused heart and on observations on anaesthetized animals after various operative procedures. In much of the experimental work digitalis has been used in amounts larger than are used therapeutically.

3 The effect of digitalis on the minute cardiac out put of man has been measured directly in two instances, and in both a significant decrease was observed.

Further studies of the cardiac out-put in man are desirable. The existing methods applicable to human subjects require intelligent co-operation, and they are at best tedious and subject to error. For this reason our present study has been carried out on dogs. It is hoped that the effect of digitalis on the cardiac out put of patients with heart disease may be studied later.

#### METHOD

The experiments were carried out on dogs in a quiet state. To avoid the exertion of struggling the animals were usually given morphine 0.012 gram per kilo of body weight. Seven experiments were done however on trained dogs that did not require morphine. One animal was used as a control, morphine alone being given. The animals were placed on their backs on an animal board.



out-put, the animals were given digifolin (Ciba) intramuscularly. The amount of the drug used varied in different experiments, according to the weight of the animal and according to whether the therapeutic or toxic effect was desired. The initial dose was never more than 0.5 cc per kilo (17 minims per 5 pounds) which represents approximately the therapeutic dose of digifolin for dogs according to Pardee (20) and which corresponds to 3 grams of the powdered leaf for a 150 pound man.

In some of the experiments this full therapeutic amount was given at the first dose. However in most instances this was given in two or three doses over a period of eight to twelve hours. In earlier experiments the effect of additional or toxic doses was also studied although in the later experiments interest centered entirely on the results obtained from the full therapeutic dose. In order to increase the margin of safety for the trained animals slightly smaller doses were used for them.

Three to five hours after the first dose of the drug the cardiac output was again determined, another electrocardiogram taken and a second dose of the drug given.

The amount used and the exact procedure varied somewhat in the different animals as shown in the tables.

Two experiments were carried out on trained animals with an increased metabolic rate, which had been previously produced by feeding thyroid gland. One experiment was performed on a dog with elevated metabolic rate after frequent bleedings. These three animals had an increased cardiac out-put at the start of the experiment and offered an opportunity to study the effect of digitalis on the hyperdynamic heart. (The effects of bleeding and of changes in metabolic rate on cardiac out-put have been studied by Blalock and Harrison and will be published at a later date.)

In two experiments on narcotized animals the usual procedure was carried out and subsequently the vagi were cut and the cardiac out-put again measured. In four experiments on trained unnarcotized animals atropine was given after digitalization and the effect on cardiac out-put determined.

In one experiment the rate of recovery from digitalis was studied by measuring the cardiac out-put every twenty-four to forty-eight hours until an approach to the normal level was found.



TABLE 2  
*Showing the effect of digitalis on dogs narcotized with morphine*

Dog number	Determination number	Data of experiments									
		Oxygen consumption cc. per min- ute	Arterial oxygen con- tent vol. per cent	Venous oxygen con- tent vol. per cent	Coefficient of utiliza- tion	Cardiac output per minute cc.	Total amount digifolin administered cc.	Hours from start of experiment	Pulse rate		
B <sub>1</sub> (6.3 kgm.)	I	54.2	17.21	13.33	3.88	1,397	0	0	54	January 16, 6.00 p.m., morphine 0.07 gram. 8.30 p.m. first deter- mination. 9.25 p.m., digifolin 2 cc	
	II	54.8	18.48	12.68	5.80	945	2.0	3	50	11.00 p.m., second deter- mination 11.35 p.m., digifolin 1 cc	
	III	63.3	16.99	11.29	5.70	1,110	3.0	5	48	January 17, 1.00 a.m., third determination. 1.30 a.m., digifolin 1 cc	
	IV	67.0	15.22	8.18	7.04	951	4.0	7	57	3.10 a.m. fourth deter- mination. 3.30 a.m., digifolin 1 cc	
	V	67.5	17.00	9.94	7.06	956	5.0	9	78	4.45 a.m. fifth deter- mination	
	VI	61.0	17.05	9.94	7.11	858	5.0	20	200	2.00 p.m., sixth deter- mination 3.50 p.m. digifolin 2 cc.	
	VII	73.6	16.78	8.39	8.39	877	7.0	22	240	6.35 p.m., seventh deter- mination 8.15 p.m., digifolin 3 cc.	
	VIII	71.8	16.53	6.58	9.95	721	10.0	25	200	9.40 p.m., eighth deter- mination 11.00 p.m., digi- folin 3 cc.	
	IX	63.0	17.54	3.74	13.80	457	13.0	28	186	January 18 12.20 a.m., ninth determination. 8.00 a.m., found dead. Autopsy No blood in pericardium. Heart con- tracted 6 cc. of blood in each ventricle	



TABLE 2—Continued

Dog number	Determination number	Data of experiments									
		Oxygen consumption cc per minute	Arterial oxygen content vol per cent	Venous oxygen content vol per cent	Coefficient of utilization L/min.	Cardiac output per minute cc.	Total amount digifolin administered cc.	Hours from start of experiment	Pulse rate		
B <sub>1</sub> (7.3 kgm.)	I	56.8	21.11	16.43	4.68	1,213	0	0	66	January 30, 12.05 p.m. control electrocardiogram 12.30 p.m., morphine 0.10 gram. 2.00 p.m., tracheotomy 3.00 p.m., first determination 3.50 p.m., digifolin 3 cc	
	II	56.5	21.74	14.67	7.07	800	3	4	54		
	III	61.7	19.80	12.32	7.48	825	3	8	186		
	IV	56.3	20.52	13.90	6.62	850	3	18	166		
	V	61.6	18.68	10.18	8.50	725	6	23	206		
	VI	51.1	20.39	11.37	9.02	566	9	28	206		
	VII	55.0	18.60	8.91	9.69	568	12	32	206		
	VIII	54.6	17.70	5.03	12.67	431	15	45	176		
B <sub>1</sub> (5.8 kgm.)	I	42.2	22.79	16.26	6.53	646	0	0	41	February 7, 12.10 a.m., control electrocardiogram 9.00 a.m., morphine 0.05 gram. 10.40 a.m., tracheotomy 11.20 a.m., first determination. 11.55 a.m., digifolin 1 cc.	



TABLE 2—*Concluded*

Dog number	Determination number	Oxygen consumption cc per min. per cent.	Atrial oxygen content vol per cent	Venous oxygen content vol per cent	Coefficient of utilization	Cardiac output per minute	Total amount digifolin administered cc	Hours from start of experiment	Pulse rate	Data of experiments
B <sub>19</sub> (10.1 kgm.)	I	116.5	15.96	9.45	6.51	1,789	0	0	66	May 8, 10 45 a.m. morphine 0.10 gram 1.30 p.m., first determination 6.00 p.m., digifolin 4 cc
	II	113.4	17.40	9.72	7.68	1,476	4	8	162	May 9, 9.05 a.m., morphine 0.10 gram. 10.00 a.m. second determination

Note The line drawn through each experiment denotes the division between toxic and therapeutic doses

produced an increase in pulse rate in six experiments. The changes in pulse rate in the morphinized animals are felt to be of less significance than the changes in the trained dogs, for morphine causes a marked slowing of the pulse, and this effect wears off before the narcotic effect is lost. In the seven experiments on trained dogs the pulse rate was diminished 18 to 24 beats per minute in four instances and was increased by 11, 22, and 100 beats per minute in the other instances, the dose of the drug not exceeding the "full therapeutic" dose in any instance.

Toxic doses caused a marked increase in pulse rate in every instance.

## 2 Oxygen consumption

This remained fairly constant in most instances. In the morphinized dogs a slight increase was noted several times as the experiment progressed. We believe this can be explained by slight restlessness as the effect of the morphine diminished. The trained dogs usually showed a decrease in oxygen consumption after digitalization. The cause of this is not clear. In seven experiments on trained dogs the oxygen consumption increased in two and decreased in five, whereas the cardiac output decreased in every instance. The maximum



decrease was 19.5 per cent in oxygen consumption and 36.2 per cent in cardiac out-put. The average change in this group was -6.1 per cent in oxygen consumption and -20.7 per cent in cardiac out-put. Changes found in cardiac out-put cannot, therefore, be attributed to variations in metabolic rate.

### *3 The arterial and venous oxygen contents*

Variations of one to two volumes per cent in arterial oxygen content were frequently encountered, and in some of the longer experiments a drop of as much as three or four volumes per cent was found. In several instances the arterial oxygen content increased, then decreased and again increased. Such changes were probably dependent on body readjustments to bleeding, with water coming into the blood stream from the tissues or red blood cells passing from the spleen into the general circulation (Barcroft (21)). The changes noted in cardiac out-put in these experiments cannot be attributed to bleeding, because Blalock and Harrison, in some unpublished observations, have found that bleeding of comparable degree produces no appreciable immediate effect on the out-put of the heart, whereas the effect on the cardiac out-put that occurs later is an increase rather than a decrease. The venous oxygen content diminished after digitalis, and this drop was greater than the arterial decline.

*The coefficient of utilization—arterio-venous difference—decreased in three instances, remained unchanged (change of less than 0.2 vol per cent) in 9 instances, and increased in 45 instances in a total of 57 determinations after digitalis.*

### *4 The cardiac out-put*

As this is the essential phase of the problem the results, which are shown in tables 2 and 3, and figures 1, 2, 3, and 4, will be analyzed in some detail. *In a total of 57 determinations after digitalis the minute out-put of the heart was increased in three instances and decreased in 54 instances.* The increases were 3, 6 and 32 per cent. The first two figures are within the limits of error of our method. The increase of 32 per cent was obtained in one determination during an experiment in which the other determinations yielded the usual decreased values for cardiac out-put. We are inclined to think that this single ex-



according to the amount of digitalis received. The maximum, minimum and average changes in cardiac out-put in each group are shown in table 7.

The values are expressed in terms of the percentage change from the control determinations. As can be seen from the figures in the last line the degree of decrease in cardiac out-put is correlated with the amount of the drug given. This is shown by figure 3.

One rather striking point is the fact that the drug in smaller doses (column I, table 7) had a very definite effect, whereas doses up to one

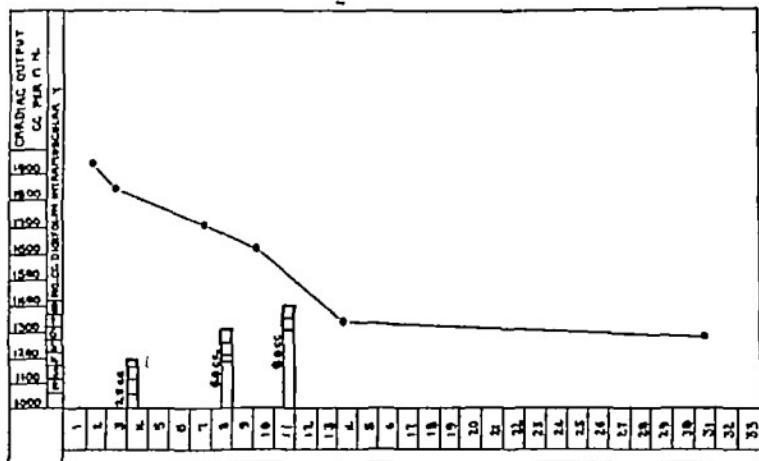


FIG. 2 THE EFFECT OF DIGITALIS IN A DOG WITH AURICULAR FIBRILLATION

The effect of increasing doses of digitalis is shown in a dog with auricular fibrillation (table 5, Dog B<sub>11</sub>). The steady decline in cardiac out put as the amount of the drug was increased is noteworthy.

and one-half times the "full therapeutic" dose did not have much more effect than did the therapeutic and sub-therapeutic doses. Figure 3 also illustrates the marked variations in the individual animals. In every instance, with the three exceptions mentioned above, the action of the drug was similar qualitatively but the quantitative effect was very variable.

The relationship between the time the drug was given and the onset and duration of the maximum effect has not been carefully studied in this work. Such observations as have been made indicate that digi-



eight days. In figure 4 an experiment (table 3, dog B<sub>17</sub>) is shown in which the "full therapeutic" dose was given and the "recovery" followed.

The *out put per beat* of the heart is a very variable factor, as has been demonstrated by practically all observers who have studied this problem in man and as has recently been demonstrated for trained unnarcotized dogs by Marshall (22) and for morphinized dogs by Harrison, Wilson and Blalock (23). Henderson, who for a long time supported the idea of a uniform stroke volume has recently come to believe that his earlier ideas were erroneous (24). There is now

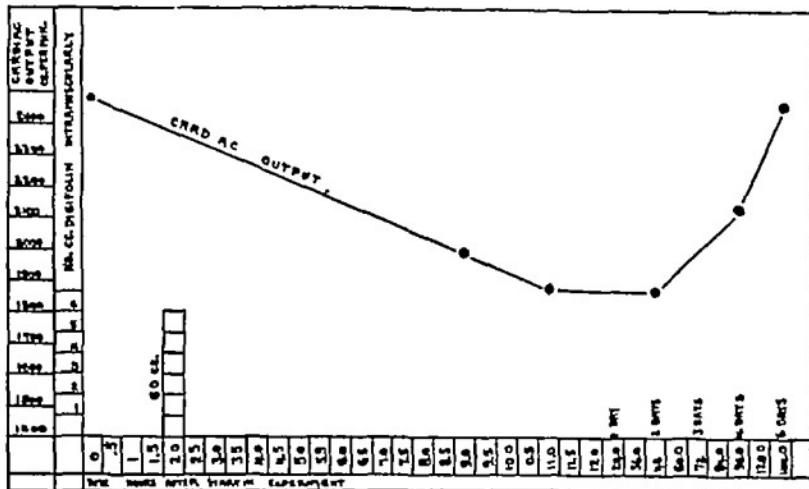


FIG. 4 THE TIME OF ONSET AND DURATION OF THE EFFECT OF DIGITALIS ON CARDIAC OUTPUT

The "recovery" was studied after a single dose of digitalis (table 3, dog B<sub>17</sub>). As can be seen the maximum decline in cardiac out-put came two days (50 hours) after the drug had been given whereas the cardiac out-put had returned to the normal level in six days (146 hours).

general concordance in the opinion that changes in cardiac out-put depend more on changes in the out-put per beat than on alteration in the cardiac rate. Our observations on digitalis are in entire support of this opinion. Digitalis produces variable effects on the pulse rate, but practically always reduces the out-put of the dog's heart, whether the pulse rate be decreased or augmented. Pulse rates are of little value in morphinized dogs because morphine is vagotonic in these



an increased cardiac out-put (+115 per cent and +69 per cent) The third animal had been subjected to excessive bleeding over a long period of time and also had an increased metabolic rate (+58

TABLE 5  
*Showing effects of digitalis in auricular fibrillation (morphinized dog)*

Dog number	Determination number	Data of experiments									
		Oxygen consumption cc. per min. sec.	Arterial oxygen content vol per cent	Venous oxygen content vol per cent	Coefficient of utilization	Cardiac output per minute cc.	Total amount digitalis administered cc.	Hours from start of experiment	Pulse rate		
B12(16 kgm.)	I 113 1	21 63	15 76	5 87	1,926	0	0	41	March 21 12 30 p.m., morphine 0.15 gram. 2 00 p.m. tracheotomy vagi isolated—not cut. 2 30 p.m., first determination		
	II 113 3	21 93	15 54	6 39	1,851	0	1	41	3 30 p.m., second determination 4 00 p.m., digifolin 3.5 cc.		
	III 100 1	22 53	16 73	5 80	1,726	3.5	6	35	7 50 p.m., third determination 8 15 p.m., digifolin 2.5 cc.		
	IV 94 0	22 22	16 38	5 84	1,609	6.0	8	41	10 30 p.m. fourth determination 11 45 p.m. digifolin 2 cc		
	V 93 4	23 28	16 38	6 90	1,355	8.0	12	158	March 22 2 30 a.m., fifth determination		
	VI 85 5	20 97	14 43	6 54	1,307	8.0	27	75	March 22 7 00 p.m. sixth determination Note Electrocardiograms showed auricular fibrillation at time of all determinations except the fifth when sinus tachycardia was present		

per cent) and increased cardiac out put (+237 per cent) In the first two animals digitalis caused no significant change in oxygen consumption and a decrease of 13 and 12 per cent respectively in cardiac out-put In the third animal the oxygen consumption was increased



atropine was given several dogs after full therapeutic doses of digitalis. The results are shown in table 6. The results were not conclusive. After vagotomy the cardiac out-put was increased in one experiment and decreased in another. After atropinization the out-put was increased slightly in one experiment and diminished by varying degrees in three instances. In none of the experiments did the cardiac out-put rise to the original level before digitalization. These results lead us to believe that digitalis affects the contraction of the heart by direct myocardial action, but we do not regard this point as definitely established.

TABLE 7  
*The relation of changes in cardiac output to the dose of digitalis*

	I Less than one-half of therapeutic dose (less than 0.25 cc. per kilogram)	II One-half to full therapeutic dose (0.25 to 0.5 cc. per kilogram)	III One to one and one-half ther- apeutic dose (0.5 to 0.75 cc. per kilogram)	IV More than one- half therapeutic doses (more than 0.75 cc. per kilogram)
Number of determinations	6	23	7	9
Highest cardiac output, per cent	+6	+32	-23	-32
Lowest cardiac output, per cent	-45	-56	-33	-67
Average cardiac output, per cent	-18	-23.3	-27.7	-46.3

### 5 *Electrocardiograms*

Frequent electrocardiograms were made during the course of each experiment. The purpose in mind was two-fold (a) to control the dosage of digifolin, by rendering certain the fact that doses considered therapeutic in quantity produced no changes in cardiac mechanism that might be interpreted as being due to serious myocardial damage, and which, in themselves, might conceivably alter the cardiac out-put, (b) to correlate, if possible, typical digitalis electrocardiographic effects with changes in the cardiac out-put. Lead II was used throughout.

A brief description of electrocardiographic results follows.

*I A* *Electrocardiographic changes observed in dogs receiving morphine followed by digifolin in quantities considered to be equivalent to or less than the therapeutic dose.* Four dogs had records made before the administration of morphine sulphate. The average amount of the drug used was 14 mgm per kilogram. After an average of 3 hours marked



*B Electrocardiographic changes following digifolin in amounts above the therapeutic dose in morphinized dogs* There were four dogs in this group, receiving digifolin in amounts varying from 0.5 to 2.1 cc per kilogram, the drug being administered in doses of 1 to 3 cc at intervals over periods of time varying from 20 to 27 hours. In every instance there was a marked increase in rate, due in three cases to the development of auricular tachycardia, with rates varying from 186 to 240 per minute, and in the remaining case to sinus tachycardia with a rate of 139 per minute. There was no constant variation in the P-R interval or in the height or sign of the T wave.

In summary, with "therapeutic" amounts of digifolin, three of seven dogs suffered significant changes in cardiac mechanism, two developing auricular tachycardias and the third an abnormal rhythm consisting of ventricular tachycardia alternating with normal sino-auricular rhythm. Four dogs were given doses greater than the therapeutic amount. Three developed auricular tachycardia, the remaining dog exhibited no significant change in cardiac mechanism. In every instance with one exception, characteristic diminution in cardiac output was obtained prior to the development of the abnormal rhythms. The P-R interval varied in either direction, and no constant changes in the T wave to indicate digitalis effect were noted.

*II Electrocardiographic changes in trained, unanesthetized dogs, following digifolin in therapeutic and sub-therapeutic doses* Of the seven dogs studied, four had decreases in rate following digifolin, varying from 10 to 25 per cent. The P-R interval in two of the four increased by 0.01 and 0.02 second respectively, while in the remaining two there was a decrease of 0.03 and 0.01 second. The T wave underwent inversion in only one of the four and this occurred simultaneously with the characteristic diminution in cardiac output.

The ventricular rate in three animals was increased, 25, 38 and 66 per cent respectively. The P-R interval in the first of the three dogs was increased by 0.01 second and in the second it was decreased by 0.03 second. The first dog showed rather frequent ventricular premature contractions, at the time the characteristic drop in cardiac output occurred. The 66 per cent increase in rate in the third animal was due to the development of a sino auricular tachycardia, rate 250. This dog, B<sub>18</sub>, three hours after two thirds of a therapeutic dose of



## DISCUSSION

In view of the fact that animals used in our experiments were entirely intact and had not been subjected to operative procedures, in view of the fact that more than one third of the experiments were carried out on trained unnarcotized healthy dogs, and in view of the uniform results obtained, we feel that there can no longer be any question as to the action of digitalis on the output of the heart of the normal dog. Digitalis diminishes the out put of the normal dog's heart.

The drug may or may not have the same effect on the diseased heart of man. In the observations quoted above by Eppinger, von Papp and Schwarz (18) a decrease of about 25 per cent in the cardiac out put occurred in a patient with valvular heart disease after a full therapeutic dose of digitalis had been given. In the other case which they studied after digitalis had been given, a patient with aortic insufficiency, the cardiac out-put was about 16 per cent less on the third day of digitalis administration than on the first day, but the relation of the action of the drug to the decrease in the cardiac out-put is not definite. On the basis of these observations one is scarcely justified in stating that the drug has the same effect on patients with heart disease as it has on normal dogs. However most drugs affect man in the same manner as the dog, most drugs act on diseased individuals and normal individuals in similar manner. Consequently we believe that it is altogether likely that digitalis diminishes the out-put of the heart in man. It is difficult to reconcile this view with the findings of Cohn and Stewart (15) who noted an increase in the ventricular excursion as judged by moving orthodiagrams after digitalization. Changes in the size of the heart shadow do not necessarily postulate similar changes in heart volume and these observers, recognizing this fact, interpreted their results as meaning that digitalis affects the contractility of the heart in man and did not claim to have demonstrated an increase in cardiac out-put.

The following discussion is based on the assumption—which is only an assumption, though seemingly a likely one—that digitalis has the same effect on the human heart as on the dog's heart. If this is granted certain points seem worthy of emphasis.

digifolin, showed a diminution of 11.2 per cent in cardiac out-put. The fast rhythm appeared 13 hours after the drug and was accompanied by a further drop of 14 per cent in cardiac out-put. However, 21 hours later, with a rate of 150 and normal mechanism, the cardiac out-put was practically the same as that at the time of the rapid rhythm.

#### NECROPSY FINDINGS

In two experiments the pericardium was found to contain blood. These experiments were discarded and are not included in our tables. The pericardial sacs of the remaining animals contained less than 5 cc or no blood. In those animals which were given toxic doses of digitalis and allowed to die from the effect of the drug, the heart was found firmly contracted and the heart chambers were free of blood. This phenomenon of systolic standstill is well recognized as a sequel of digitalis poisoning. The hearts of the other animals—those sacrificed with ether after receiving sub-lethal doses of digifolin—appeared to contain less blood than the hearts of normal dogs and the ventricular walls were firm. We gained the impression that these hearts were somewhat smaller than the hearts of dogs which had received no digitalis, but of this we have no proof. It was noted, however, in many of the experiments, that ventricular punctures which could be carried out with ease in the control observations, became increasingly difficult as digitalis was administered, and this was attributed to a diminishing size of the ventricular cavities. We frequently found it necessary to use a long needle to enter the ventricle of the digitalized dog whereas a short needle had sufficed for the control puncture.

X-ray studies were not made of the heart size. These would have been desirable. In their absence it is felt that the above observations, taken together with the findings of Strong and Gordon (13) (diminished size of the rabbit's heart after strophanthin), and Levy (14) (diminished size of the heart of patients with pneumonia after digitalis) indicate that digitalis both in the therapeutic and toxic doses diminishes the volume of the heart. We agree with Schmoll (7) that the essential action of digitalis is concerned with an increased tonicity of the heart. The significance of this conception is discussed below.

## DISCUSSION

In view of the fact that animals used in our experiments were entirely intact and had not been subjected to operative procedures, in view of the fact that more than one third of the experiments were carried out on trained unnarcotized healthy dogs, and in view of the uniform results obtained, we feel that there can no longer be any question as to the action of digitalis on the output of the heart of the normal dog. Digitalis diminishes the out-put of the normal dog's heart.

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The following discussion is based on the assumption—which is only an assumption, though seemingly a likely one—that digitalis has the same effect on the human heart as on the dog's heart. If this is granted certain points seem worthy of emphasis.

*The sedative action of digitalis*

The work of the heart depends on numerous factors of which the most important are, (a) the cardiac out-put, (b) the blood pressure, (c) the blood viscosity, (d) the elasticity of the blood vessels and (e) the total volume of circulating blood. In our experiments the last three factors may be considered as constant. Blood pressure was not measured but it is generally recognized that digitalis in therapeutic doses has no significant effect on blood pressure either in man or in the experimental animal.

Since digitalis has no significant effect on the other factors entering into the work of the heart and since it diminished the amount of blood pumped by the heart, the drug may be asserted to diminish the work of the heart and in this sense is to be regarded as a cardiac sedative. It is probable that this sedative action is more marked in diseased than in normal hearts as will be pointed out later.

The conception of digitalis as a cardiac sedative is contrary to current opinion. The drug has been generally considered by clinicians as a "cardiac tonic" despite the conflicting experimental evidence upon which this idea is based.

From the point of view of the sedative action, digitalis may be considered as affecting the circulation in the same way as morphine affects the respiration. The value of morphine in the various forms of severe dyspnea of respiratory origin is unquestioned. Morphine is known to be a respiratory sedative, and in view of our experiments we believe that digitalis acts on the circulation in a similar manner—by resting an over-strained mechanism. The evidence that digitalis decreases the work of the dog's heart is conclusive and by analogy to morphine the evidence that the drug decreases the work of the diseased human heart becomes very suggestive.

In pneumonia the respiratory minute volume is increased. Harrison and Blalock (25) have recently shown that the circulatory minute volume is also increased in dogs with this disease, and Leegaard (26) found the same result in rabbits. For many years the value of morphine in pneumonia has been considered as questionable, because the "depressing" effect on respiration was feared. This fear has been largely overcome and morphine has come in the last few years to

occupy an important place in the treatment of pneumonia (27) (28) (29) Similar uncertainty has existed concerning the value of digitalis in pneumonia (30), although Cohn and Jamieson (31) showed that digitalis has the same effect in patients with pneumonia as in patients without pneumonia, and Levy (14) demonstrated a decrease in the size of the heart after digitalization in pneumonia. The explanation for the lack of favorable results in pneumonia from digitalis may lie in the fact that the drug is usually not given at all or is given in small doses for the first few days of the disease and then "pushed" when "signs of circulatory failure" develop. In view of the conception of digitalis as a cardiac sedative it would seem that digitalis has been incorrectly used in pneumonia. If given at all the drug should be given early in the disease and in full therapeutic doses, for it is early in the disease that the cardiac out-put is probably increased—if the human heart reacts to pneumonia in the same manner as the heart of the dog and rabbit—and "circulatory failure" in pneumonia is probably at least partially dependent on pre-existing strain from increased out-put over a period of several days. We believe that digitalis is contraindicated in pneumonia when outstanding evidence of "circulatory failure" is present, as it is likely then under this condition the out put of the heart is already much diminished. Digitalis should be used to prevent and not to combat "circulatory failure" in pneumonia.

There is therefore rational experimental evidence to believe that digitalis given early and freely may be of considerable value in pneumonia, whereas its value late in the disease is questionable, and quite possibly the drug is harmful under these conditions.

The tonic action of digitalis has been considered for years as the essential feature of the action of the drug. This is true in one sense—but in a different sense from the usual idea of "tonic" action. Digitalis has been generally considered to increase the contractility and consequently the out put of the heart. The drug has just the opposite effect in the dog and probably in man. However, there is evidence to indicate that digitalis in therapeutic doses increases the tonicity of the heart muscle. Strong and Gordon (13) found a diminution in the size of the heart shadow in normal rabbits and rabbits with experimental myocarditis after administration of strophanthin. Levy (14)

reported a similar effect from digitalis in patients with pneumonia. Systolic "stand-still" is the generally recognized effect of lethal doses and we have referred above to our difficulty in puncturing the ventricles after digitalization.

Schmoll (7) in 1911 claimed that the essential action of digitalis was the effect on tonicity. In the light of our observations we agree with Schmoll and believe that digitalis acts on the heart by increasing the tonus of the muscle, the diminished contractility being a secondary effect and being dependent on a diminished diastolic relaxation. In this sense digitalis is a "cardiac tonic." The term is obviously misleading, as it may denote either augmented contractility or augmented tonicity and these are functionally opposite effects.

#### *The action of digitalis in heart disease*

It has been almost universally assumed that such symptoms as dyspnea and orthopnea and such signs as cyanosis, edema and engorgement of the liver and lungs, are dependent on diminished cardiac out-put and the object of treatment has been believed to be an increase in out-put. Considerable evidence has been accumulated against this view. Thus the ideas of fifty years ago as to the value of exercise have been completely abandoned, as Pratt (32) has pointed out. Henderson and Haggard (33) have recently shown that the out-put of the heart is less when a person is sitting than when lying down. Field and Bock (34) have confirmed this finding and have indicated the significance of this observation from the viewpoint of the interpretation of orthopnea. The latter authors regard orthopnea as a protective mechanism, the patient being more comfortable in the sitting position, because the cardiac out-put is diminished. Everyone who has watched many cardiac patients has noted that some of them, when in great distress, hang their feet over the side of the bed, and this, as the above investigators have shown diminishes the out-put still more. This leads us to the conclusion that those measures which diminish the out-put of the heart are beneficial in cardiac insufficiency and this conclusion constitutes one of the strongest links of evidence that digitalis diminishes the out-put of the insufficient human heart, as well as the out-put of the normal dog's heart.

Furthermore there is reason to believe that the cardiac out-put is

not diminished in all cases of cardiac insufficiency Eppinger, Kisch and Schwarz (35) report observations on the cardiac out-put of a patient with mitral disease, who had a minute out put of 5 15 liters when "fully compensated" and a year later when decompensated" the minute out put was 10 13 liters Eppinger, von Papp and Schwarz (18) found a great increase in minute out-put in patients with acute pulmonary edema (cardiac asthma) The same investigators found a normal or low coefficient of utilization of oxygen in a large percentage of their cases of mitral and aortic disease with "decompensation," indicating that the cardiac out-put was probably not diminished These investigators are inclined to stress the importance of the peripheral circulation in the production of cardiac insufficiency and believe that the essential factor in cardiac failure is a lack of functional balance between the two ventricles rather than diminished cardiac out-put This conception explains why some patients have congestion of the lungs without congestion of the systemic circulation and why other patients have edema of the extremities and engorgement of the liver without râles in the lungs—facts which are difficult of explanation on the basis of an assumption that diminished cardiac out-put and consequent general slowing of the blood stream are the essential factors in producing the signs of decompensation These two theories of the mechanism of cardiac insufficiency are almost diametrically opposed The one theory states that the important feature is the "level" (or amount) of cardiac out put, the other asserts that it is the "balance" or coordination of the various circulatory functions that is the essential factor in maintaining circulatory efficiency

Our findings in regard to digitalis are particularly compatible with the latter conception If "decompensation" is primarily due to the fact that one ventricle is unable to keep pace with the other, then anything which diminishes the total cardiac out put should tend to reestablish the balance at a lower level This is what digitalis apparently does—protects the weaker ventricle from overstrain by the stronger

In cardiac decompensation with relative mitral or tricuspid insufficiency digitalis may cause increased tone of the auriculo-ventricular rings It is possible that the drug lessens regurgitation by this means, and if so the efficiency of the heart is increased, while the work is

decreased This conception may explain the well recognized fact that the digitalis is more beneficial in mitral incompetence than in aortic incompetence Relative aortic insufficiency is very rare and one would expect more benefit in those cases in which the mechanical defect is diminished

Our findings have led us to believe that digitalis has two general indications (a) *To combat cardiac insufficiency and (b) to prevent cardiac insufficiency* The first of these indications is too well recognized to require further comment, but the second merits some discussion

*There are certain conditions in which there is evidence that the cardiac work is increased* Pneumonia has already been mentioned Blalock and Harrison (36) have observed an increased cardiac out-put in chronic anemia in dogs, after successive bleedings Robinson and Burwell (37) have found a great increase in cardiac out-put in a patient with hyperthyroidism Davies, Meakins and Sand (38) report several observations of increased out-put in patients with this disease Odaira (39), working with rabbits, and Blalock and Harrison (40) working with dogs found increased cardiac out-puts after feeding thyroid substance Blalock, Harrison and Wilson (41) found increased cardiac out-put in experimental partial respiratory obstruction, whereas Harrison, Wilson and Blalock (25) found increased cardiac out-put in experimental acidosis Harrison and Blalock (42) have recently observed a great increase in cardiac out-put in experimental anoxemia Blalock (43) has noted increased cardiac out-put in dogs under ether anesthesia Harrison, Dock and Holman (44) found increased cardiac out-put in experimental arterio-venous fistulae There are possibly many other conditions, yet to be investigated, in which the out-put of the heart and consequently the strain on the heart, are increased In many or all of these conditions the increased cardiac out-put is a compensatory mechanism, which may in itself be a source of danger by overstraining the heart to the point of failure or possibly by producing a lack of circulatory balance We have found that digitalis decreases the out-put of the "hyperactive" hearts of thyrotoxic and anemic dogs (table 4) There is therefore a rational experimental basis for believing that this drug may be of value in preventing cardiac failure from overstrain in these conditions If

given to patients suffering from any of the above conditions digitalis should be given early, before evidence of circulatory failure develops (See discussion on pneumonia above) The experimental evidence is sufficient to warrant a thorough trial of digitalis in patients suffering from the above conditions the final answer as to whether digitalis is valuable in such instances must rest upon carefully controlled clinical observations of many well studied cases

#### *Contraindications for digitalis*

The drug is widely used in all forms of acute and chronic circulatory failure from all causes, and much uncertainty exists as to its value in many groups of patients Our results lead us to believe that digitalis is contraindicated whenever evidence of diminished cardiac out-put exists without simultaneous evidence of lack of ventricular balance In the absence of extensive studies on cardiac out-put in man these criteria are difficult to apply We can only interpret such evidence as is available and hold our interpretation open to change in the light of newer advances To be more specific in the absence of local causes, we may consider congestion of the liver, lungs or extremities, ascites and hydrothorax as evidences of lack of ventricular balance and as indications for digitalis regardless of whether cardiac out-put is believed to be decreased, increased or normal When none of these signs are present, a feeble, rapid, regular pulse associated with faint heart sounds, with or without dyspnea, orthopnea and cyanosis may be regarded as evidence of diminished cardiac out-put and as a contraindication to digitalis Such conditions are found in surgical or traumatic shock, after prolonged anesthesia—particularly in elderly persons, in acute peritonitis, in the terminal stages of most acute infectious diseases, including lobar pneumonia, and in patients moribund from any cause Digitalis is widely used in such cases because of its supposed "tonic" action on the heart The evidence presented in this paper suggests that when so used the drug may be harmful As a matter of fact when digitalis is given to these patients it is often administered intramuscularly as digifolin in doses of one to three cubic centimeters a day, and such doses probably have no effect at all If the drug were administered in full amount in such cases we should probably see harmful effects occasionally As it is one does not usually observe any effect from digitalis in these patients

## SUMMARY

The cardiac out-puts of morphinized dogs and trained unnarcotized dogs have been studied by the Fick method before and after the intramuscular administration of digifolin. The following results have been obtained.

1 The calculated "full therapeutic dose" of the drug caused an average decrease of approximately 25 per cent in the cardiac output per minute.

2 Smaller doses than the above caused a definite but smaller decrease and larger doses a greater decrease in the cardiac out-put per minute.

3 Although individual dogs showed marked variations in the quantitative effect of the drug on cardiac out-put, the qualitative effect (diminution in minute out-put) was similar in all the dogs studied.

4 The maximum effect of a single dose became manifest in about six hours from the time of administration, and lasted for about forty-eight hours. The cardiac out-put then gradually increased, reaching the normal level in approximately six days.

5 One dog with auricular fibrillation responded to digifolin by a decrease in cardiac out-put, slightly but not significantly greater than the decrease found in the animals with normal mechanism.

6 Three dogs with increased cardiac out-put showed an average decrease of 14 per cent after digifolin.

7 The effects of vagotomy and atropinization of previously digitalized dogs were variable. The cardiac out-put did not return to the normal level in any instance.

8 Although the changes in minute cardiac out-put were uniform, the changes in pulse rate, in oxygen consumption, in out-put per beat, and in the electrocardiograms were variable.

9 Evidence has been presented to show that the results obtained after digitalization are probably primarily dependent on an increased tonicity of the heart muscle.

In order to consider the clinical application of the above findings it has been necessary to assume, without definite proof that digitalis affects the diseased human heart in the same manner as it affects the normal dog's heart. Considerable evidence for this hypothesis

has been presented. On the basis of this assumption the following points are emphasized:

1 Digitalis is a cardiac sedative in one sense—it diminishes cardiac out-put and hence diminishes cardiac work. The effect of digitalis on the circulation is considered as somewhat analogous to the effect of morphine on the respiration.

2 Digitalis is a cardiac tonic in another sense—it increases tonicity and diminishes dilatation.

3 The action of digitalis furnishes evidence for the idea that a failure in ventricular balance is an important factor, if not the most important factor, in the production of the symptoms of cardiac insufficiency. This is opposed to the conception of cardiac failure as being dependent primarily on a general diminution of the out-put of the heart.

4 The beneficial action of digitalis in cardiac insufficiency with normal rhythm is to be attributed to two factors (*a*) the effect on contractility—diminished work, and (*b*) the effect on tonicity—increased cardiac efficiency from decreased regurgitation and diminished dilatation. It is believed that digitalis has a more striking effect on the work of the decompensated heart than on the work of the normal heart.

5 No definite correlation has been found to exist between changes in the cardiac mechanism as revealed by electrocardiograms and changes in cardiac out put.

6 The indications for digitalis are believed to be:

*a* To combat cardiac insufficiency. This is in agreement with general clinical experience.

*b* To prevent cardiac insufficiency in patients in whom the heart is subjected to the strain of prolonged, increased work. Examples are (1) mechanical defects, as valve lesions, (2) increased systemic or pulmonary blood pressure—as hypertension or asthmatic bronchitis, (3) prolonged increased cardiac out-put—as hyperthyroidism, pneumonia, anemia, anoxemia, etc. The clinical evidence for the value of digitalis in these cases is not conclusive.

9 The contraindications for digitalis may be summarized as those conditions in which the clinical picture indicates acute circulatory failure or shock, without evidence of visceral or peripheral congestion.

When the cardiac out-put is believed to be materially decreased and simultaneous evidence for lack of ventricular balance does not exist, digitalis, if given in effective doses, may be expected to have a harmful action

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#### BIBLIOGRAPHY

- 1 Robinson, G Canby *The Therapeutic Use of Digitalis* Baltimore, 1923
- 2 Cushny, A R *The Action and Uses in Medicine of Digitalis and its Allies* London, 1925
- 3 Gottheb, R , and Magnus R *Arch f Exp Path u Pharm*, 1903, li, 30 *Digitalis und Herzarbeit*
- 4 Magnus, R , and Sowton, S C M *Arch f Exp Path u Pharm*, 1910, lxii, 255 *Zur Elementarwirkung der Digitaliskörper*
- 5 Cushny, A R (2), p 118
- 6 Cushny, A R (2), p 113
- 7 Schmoll, Emile *Amer Jour Med Sc*, 1911, cxli, 35 *The Tonic Use of Digitalis*
- 8 Gottheb, R Quoted by Robinson (1), p 43
- 9 Schmiedeberg Quoted by Robinson (1), p 43
- 10 Cushny (2), p 117
- 11 Cohn, A E *Jour Amer Med Assoc*, 1915, lxv, 1527 *Clinical and Electrocardiographic Studies on the Action of Digitalis*
- 12 Cohn, A E , and Levy, R L *Proc Soc Exp Biol and Med*, 1920, xvii, 160 *The Effect of Therapeutic Doses of Digitalis on the Contraction of Heart Muscle*
- 13 Strong, G F , and Gordon, Burgess *Arch Int Med*, 1923, xxxii, 510 *Studies on the Rabbit's Heart. I Effect of Strophanthin on the Size of the Normal and of the Abnormal Heart*
- 14 Levy, R L *Arch Int Med*, 1923, xxxii, 359 *Size of the Heart in Pneumonia A Teleroentgenographic Study, with Observations on the Effect of Digitalis Therapy*
- 15 Cohn, A E , and Stewart, H J *Jour Clin Invest*, 1924, i, 97 *Evidence that Digitalis Influences Contraction of the Heart in Man*
- 16 Vagt Quoted by Cushny (2), p 159
- 17 Kauffman, Rudolph *Zeitsch f Exp Path u Ther*, 1913, xii, 165 *Ueber den Einfluss des Schmerzes u der Digitalis auf die Herzarbeit des normalen Menschen*

- 18 Eppinger, Hans, von Papp, L , and Schwarz, H. Über das Asthma Cardiale. Berlin, 1924, p 191
- 19 Van Slyke, D D , and Neill, J M Jour Biol Chem., 1924, lx, 523 The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement.
- 20 Pardee, H. E B Jour Amer Med Assoc., 1925, lxxxv, 1359 Hypodermic Digitalis Preparations.
- 21 Barcroft, S Jour Physiol., 1925, ix, 443 A Contribution to the Physiology of the Spleen
- 22 Marshall, E K , Jr Amer Jour Physiol., 1925, lxxii, 192 Cardiac Output
- 23 Harrison, Tinsley, R , Wilson, Charles P , and Blalock, Alfred Jour Clin Invest., 1925, R, 547 The Effects of Changes in Hydrogen Ion Concentration on the Blood Flow of Morphinized Dogs.
- 24 Henderson, Yandell Lancet, 1925, ii, 1317 Efficiency of Heart and Its Measurement.
- 25 Harrison, Tinsley R , and Blalock, Alfred Jour Clin Invest., 1926, ii, 435 Cardiac Out-put in Pneumonia in the Dog
- 26 Leegaard, T Skand Arch f Physiol., 1925, xlvi, 323 The Output of the Heart in Experimental Pneumonia.
- 27 Cole, Rufus Nelson Loose Leaf Living Medicine, vol. i, p 258 Treatment of Acute Lobar Pneumonia.
- 28 Lord, Frederick T Ibid., p 305 Bronchopneumonia.
- 29 Austrian, Charles R Tice's Practice of Medicine, iii, 62 New York.
- 30 Irons, Ernest E Oxford System of Medicine, iv, 855 New York. Acute Lobar Pneumonia
- 31 Cohn, A. E , and Jamieson, R A. Jour Exp Med., 1917, xxv, 65 The Action of Digitalis in Pneumonia.
- 32 Pratt, Joseph H South Med Jour., 1920, xiii, 481 Rest and Exercise in the Treatment of Heart Disease
- 33 Henderson, Yandell, and Haggard, H W Amer Jour Physiol., 1925, lxxii, 193 The Circulation and Its Measurement
- 34 Field, H Jr , and Bock, A V Jour Clin Invest., 1925, ii, 67 Orthopnea and the Effect of Posture upon the Rate of Blood Flow
- 35 Eppinger, Hans, Kisch, Franz, and Schwarz, Heinrich Klin Woch., 1925, iv, 1101 Arbeit und Kreislauf
- 36 Blalock, Alfred, and Harrison, Tinsley R Unpublished Observations on the Cardiac Output in Anaemia.
- 37 Robison, G Canby, and Burwell, C Sidney Personal communication
- 38 Davies, J W , Meakins J , and Sands, J Heart, 1924, xi, 299 The Influence of Circulatory Disturbances on the Gaseous Exchange of the Blood V The Blood Gases and Circulation Rate in Hyperthyroidism.

- 39 Odaira, T Tohoku Jour Exp Med , 1925, vi, 325 Studien über Gasstoffwechsel und minutenvolum I Die Beziehung des Gasstoffwechsels und minutenvolums zur inneren Sekretion
- 40 Blalock, Alfred, and Harrison Tinsley R Unpublished Observations on the Cardiac Output in Experimental Hyperthyroidism
- 41 Blalock, Alfred, Harrison, Tinsley R , and Wilson, Charles P Arch of Surg , 1926, xii, 81 The Effect of Partial Tracheal Obstruction on the Circulation and Respiration of Morphinized Dogs
- 42 Harrison, Tinsley R , and Blalock, Alfred Unpublished Observations on the Effect of Anoxemia on Cardiac Output
- 43 Blalock, Alfred Personal communication
- 44 Harrison, Tinsley R , Dock, William, and Holman, Emile Heart, 1924, xi, 337 Experimental Studies on Arterio-Venous Fistulae, Cardiac Output

## STUDIES IN SERUM ELECTROLYTES

### I CONCENTRATION OF ELECTROLYTES AND NON-ELECTROLYTES IN THE SERUM DURING LOBAR PNEUMONIA

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It has long been known that during the course of lobar pneumonia alterations in the chloride distribution in the blood and urine occur. During the stage of active infection there is a retention of chloride in the body as manifested by a decreased chloride elimination, whereas the blood in this period shows a hypochloremia. The present study was designed to follow the changes in the concentration in the serum of the electrolytes in general and of the non-electrolytes in patients with lobar pneumonia.

Hutchison (1898) studied the chloride metabolism in pneumonia and reviewed the literature up to 1898. He showed that diminution in the urinary chlorides might occur in other febrile conditions but was not as constant as in pneumonia and did not occur in other pulmonary diseases. For this reason he believed that an examination of the chlorides was of value in differentiating pneumonia from empyema, pleurisy, et cetera.

Peabody (1913) studied the inorganic metabolism in pneumonia and reviewed the literature. In eight cases of pneumonia in which he studied the relation of chloride changes in the blood to changes in the urinary excretion he found that the decrease in the chloride excretion was associated with a lowered chloride concentration in the blood, and that at the time when excretion was increased, the blood chloride concentration was raised. His results also indicated a lowering of calcium and magnesium concentration of the blood during the stage of active infection.

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McLean (1915), Maver and Schwartz (1916), Killian (1921), and Haden (1925) also have found a lowering of the chloride concentration in the blood serum in pneumonia. Maver and Schwartz (1916) observed that a diminished concentration of the plasma chlorides occurred at the height of the fever and that an increased concentration occurred immediately after the crisis. Peters, Bulger, Eisenman, and Lee (1926b) believe that factors in determining the low serum chloride in pneumonia are in some cases diminution in base and in others the presence of an unusual amount of organic acid.

Gerstenberger, Burhans, Smith, and Wetzel (1923) in studying the inorganic phosphorus and calcium concentration in the blood of non-rachitic children suffering with pneumonia found a reduction in both before the crisis. Jansen (1924) believes the calcium concentration is lowered during the febrile period and is increased in the afebrile.

Gram (1923) studied the freezing point depression on various pathological sera and found a decreased freezing point depression in nine determinations made during the active infection in lobar pneumonia. The average freezing point depression in his pneumonia serum observations was  $0.534^{\circ}\text{C}$ . He made no post-critical determinations.

Considerable work is recorded in the literature on the blood gases in pneumonia. Peabody (1912) reviewed the literature on the carbon dioxide content of the blood. In his studies he concluded that there was a decrease for the most part in pneumonia but that "the diminution in the carbon dioxide in the blood bears no immediate relation to the temperature, as it may persist for some days after the patient is afebrile."

Stadie (1919) and Stadie and Van Slyke (1920) showed that neither the arterial nor venous carbon dioxide values were increased above the normal in pneumonia.

Meakins (1921) found the carbon dioxide content of arterial blood in four cases of lobar pneumonia decreased to from 42 to 44 volumes per cent (18.7 to 19.6 millimoles per liter). He believed that this decrease was the result of increased pulmonary ventilation.

Hastings, Neill, Morgan, and Binger (1924) studied the blood gases and reaction in sixteen cases of pneumonia. The carbon di-

oxide content was within the normal area in twenty-four determinations, in three analyses the values were high and in three they were low

Peters, Bulger, Eisenman, and Lee (1926) call attention to the fact that while the CO<sub>2</sub> content in pneumonia cases in which the normal limits for afebrile patients, it is higher than that of patients with other febrile infections

#### MATERIAL AND METHODS

Sixty-two specimens of blood from twenty-two patients with typical lobar pneumonia on the service of Dr G W Norris at the Pennsylvania Hospital were examined

*Bleedings* Blood was drawn by vein puncture before breakfast and collected under oil. An eighteen caliber needle was introduced into the largest vein procurable at the elbow so that the blood would flow freely and venous stasis would be lessened. Peters, Bulger, Eisenman, and Lee (1926a) have shown that prolonged venous obstruction may alter the blood constituents. The blood was defibrinated under oil, centrifuged, and the serum removed under oil according to the technique of Austin and Gram (1924). Whenever possible, bleedings were made at intervals of two or three days on each patient before and after the crisis.

*Freezing Point* In the determination of the freezing point the cryoscope of Burian and Drucker (1909) was employed. This instrument was checked at frequent intervals by making control readings of the depression in freezing point of solutions of known concentrations of sodium chloride. Before every two or three single determinations the zero reading of the Beckman thermometer was found by taking the freezing point of distilled water. Two or more readings were always made on separate samples of a given specimen of serum and the average of these readings was taken. In order to obtain an accuracy of plus or minus 0.005°C, several precautions were deemed necessary: the cooling mixture was not lower than -5°C, mechanical methods for stirring at a slow constant rate were utilized, and the convergence temperature was not more than 0.3°C below the freezing point obtained.

*Conductivity* The conductivity estimations were made with the

Christiansen (1922) ionometer as employed by Gram and Cullen (1923) using 110 volts D C. This instrument was standardized daily by means of a known sodium chloride solution. Several readings of each specimen of serum were made at 20°C and expressed in sodium chloride equivalents, i.e., in terms of the concentration of sodium chloride in water required to give the same conductivity. In order to correct the conductivity for protein we used the correction of Gram and Cullen (1923) which is based upon the refractive index of serum against distilled water using the Abbe refractometer with a constant temperature of 17.5°C.

$$C_c = C_o \frac{100}{100 - (2.2 \times Pr)}$$

$$Pr = \frac{R_o - R_w - 0.0028}{0.00172}$$

where

$C_c$  = corrected conductivity in NaCl equivalents

$C_o$  = observed conductivity in NaCl equivalents

$P_r$  = protein, grams per cent

$R_o$  = refractive index of serum (Abbe refractometer)

$R_w$  = refractive index of water (Abbe refractometer) = 1.3332

0.0028 = arbitrary correction for salts and non-electrolytes in serum

0.00172 = Reiss' figure for the refractive index of 1 per cent protein solution

*Dry weight* As a means of checking the percentage of protein determined refractometrically, dry weights of the sera were obtained. Filter paper was folded into a weighing flask and dried at 110°C to a constant weight. One cubic centimeter of serum was dropped on the paper and weighed. The serum was then dried at 110°C and weighed two or more times until constant values were obtained.

The *total base* of fifty one samples of blood serum were analyzed according to the method of Stadie and Ross (1925). The *chlorides* were determined by means of the Van Slyke method (1923). The method of Folin (1922) was used in the determination of *blood urea nitrogen*, *sugar*, *creatinine*, and in several instances *non-protein nitrogen*.  $CO_2$  analyses were made with Van Slyke's technique (1921).

In the determination of the blood *cholesterol* the Myers and Wardell method was used (see Myers 1924)

#### RESULTS

We will present our results not only by the individual case tabulation (see table 2) but also by the aggregate of each type of determina-

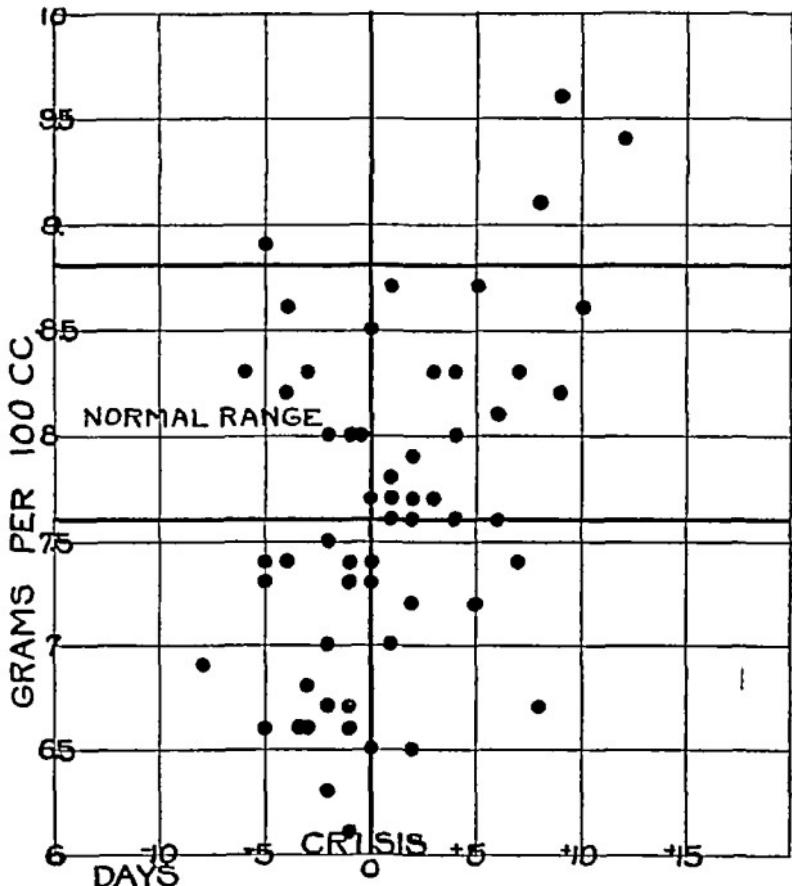


FIG 1 TOTAL SERUM PROTEIN (REFRACTOMETRIC)

tion as plotted before and after the crisis. The crisis is taken as the zero day, the time of the active infection or febrile period is designated minus days, and the time after the crisis is designated as plus days.

*Protein* If the normal values of protein in serum are taken as 7.6 to 8.8 per cent (Gram (1924) and table 1) it will be seen that the protein percentages apparently fluctuate considerably in the same individual. However, in the febrile period there is a tendency for the total serum protein to be lower than normal. This is followed about the time of the crisis by a tendency toward the normal range.

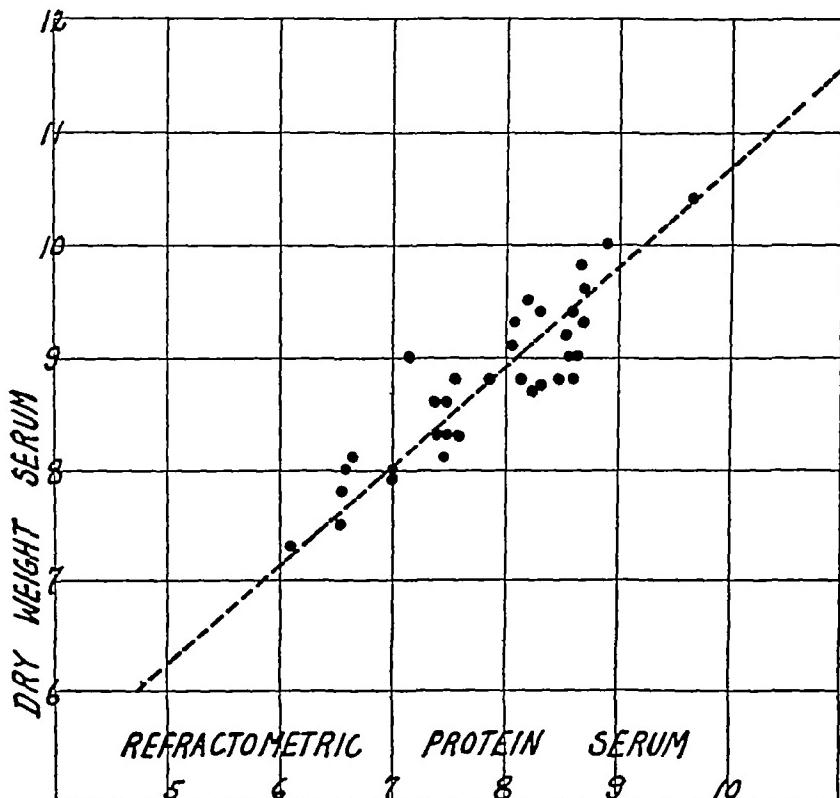


FIG 2 SERUM DRY WEIGHT—SERUM PROTEIN  
Expressed in grams per 100 cc

or in several instances to above normal (fig 1). Increase and decrease in protein was accompanied by and closely paralleled respective increase and decrease in the dry weights. In our series the percentage of protein present as determined by means of the refractometer and Reiss' formula (Domarus, 1921) was approximately ninety per cent of the dry weight (fig 2).

*CO<sub>2</sub> content* CO<sub>2</sub> content of the serum in our series remained for the most part within normal limits. In the period of hyperpyrexia seven analyses were below normal and in all of these there was hyperpnea (fig 3).

*Corrected conductivity* A range of corrected conductivity between (130 and 139) milliequivalents per liter has been taken as the normal (fig 4, table 1 and Gram (1924)). Before the crisis the corrected con-

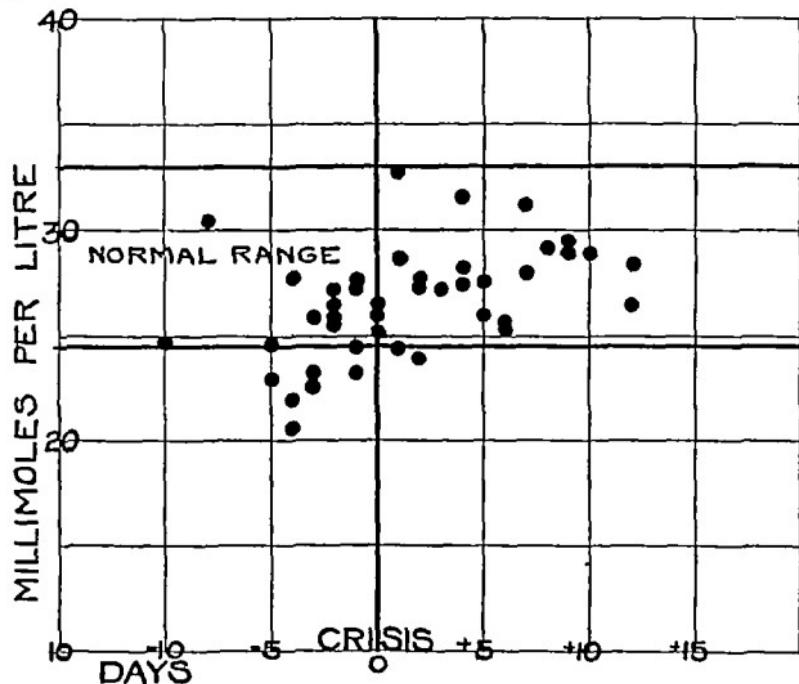


FIG 3 CO<sub>2</sub> IN SERUM

ductivity was consistently below the normal level. The decrease existed even in the earliest stages of the infection studied and in a given individual persisted immediately up to the crisis. With the exception of one case, in which the crisis occurred on the fifth day of the disease, the lowest conductivity was not observed earlier than the fifth day. In four out of eight cases which were followed for four days or more before the crisis there was a further fall in conductivity between the fifth and ninth days of the disease. Therefore, while

conductivity was below normal in the earliest studies made, the fall may be progressive up to the ninth day. With the advent of the crisis, however, the conductivity rose toward the lower normal limit and sometimes continued to rise for several days after the crisis.

*Total base.* The analyses show a satisfactory correlation with the corrected conductivity values. If the base values be plotted as abscissae and the corrected conductivity values be plotted as ordi-

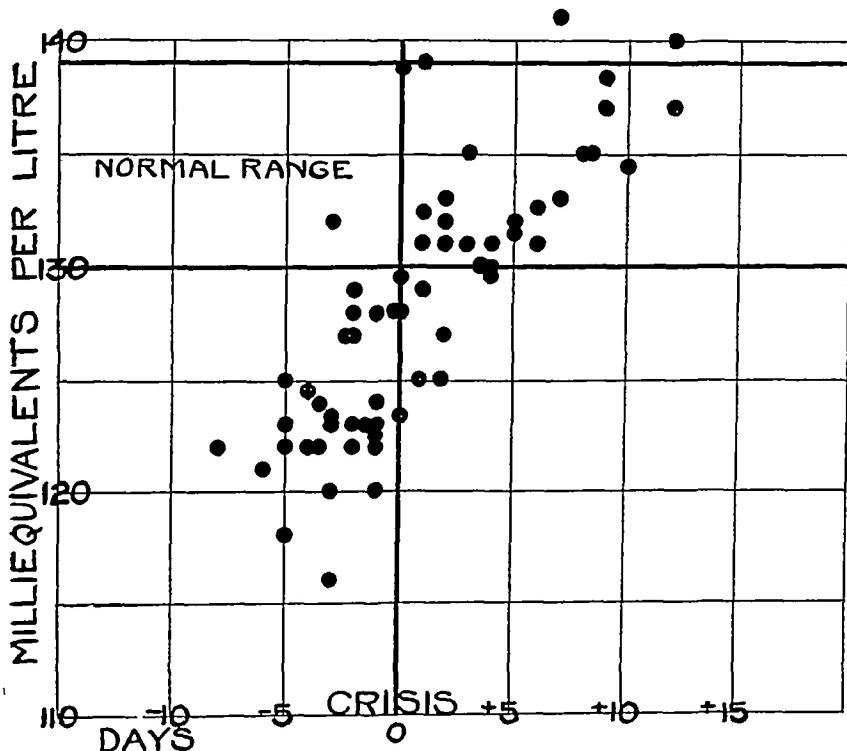


FIG 4 CORRECTED CONDUCTIVITY OF SERUM (NaCl EQUIVALENTS)

nates it is shown on figure 5 that these values fall on a diagonal line and, using the factor of Gram and Cullen for obtaining corrected conductivity, it is approximately true that 1.13 times corrected conductivity equals total base ( $1.13 C_e = \text{base}$ ). Because of this linear relationship it is believed that the corrected conductivity may be used as a means of approximating the total base in serum where an accuracy within the limits of plus or minus 3 per cent is adequate.

Since we have shown that the total serum base is approximately proportional to the conductivity, it is clear that the total base concentration of the serum was decreased throughout the entire pre-clinical course of infection and tended to become normal after the crisis (fig. 6)

*Chlorides* The chloride concentration in the blood serum during the course of lobar pneumonia was constantly below the normal level

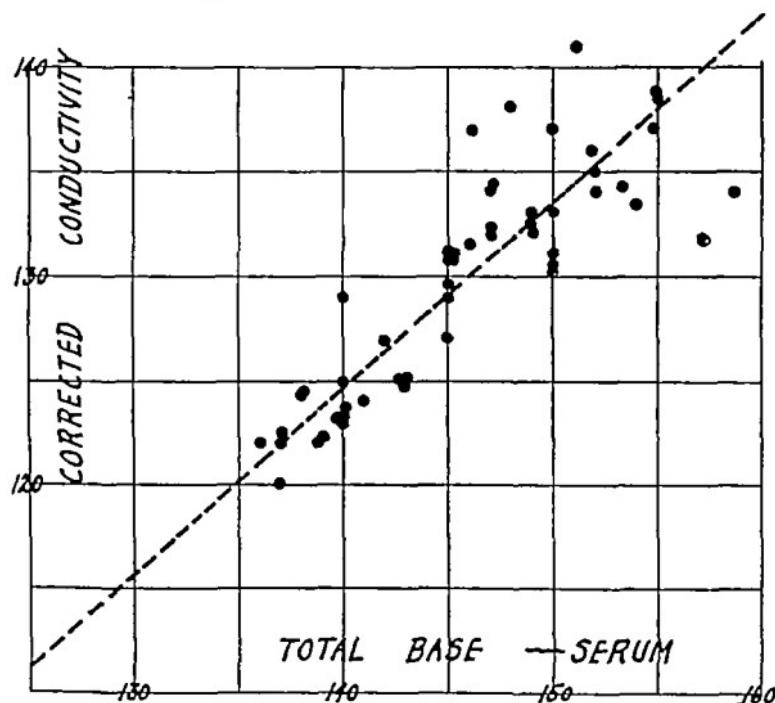


FIG 5 RATIO OF CORRECTED CONDUCTIVITY OF SERUM TO TOTAL SERUM BASE  
(113 C. = base) (milliequivalents per liter)

(fig. 7) Following the crisis there was a tendency for a return to the lower normal range but in ten instances this was delayed. Haden (1925) has suggested a possible relation between the disturbance of body chlorides and the toxemia of lobar pneumonia. We have been unable to find any correlation clinically between those cases which showed an especially marked decrease of chlorides or the decrease persisting after the crisis and the severity of the toxemia.

*Freezing point* The depression in freezing point is dependent on the total os-molar concentration of solutes in the serum and is dependent therefore, not only on electrolytes, but on non-electrolytes as well. Normally the freezing point depression is between  $0.55^{\circ}$  and  $0.57^{\circ}\text{C}$  (fig 8 and table 1). In our series the freezing point depression was normal (two observations) or diminished in all of the

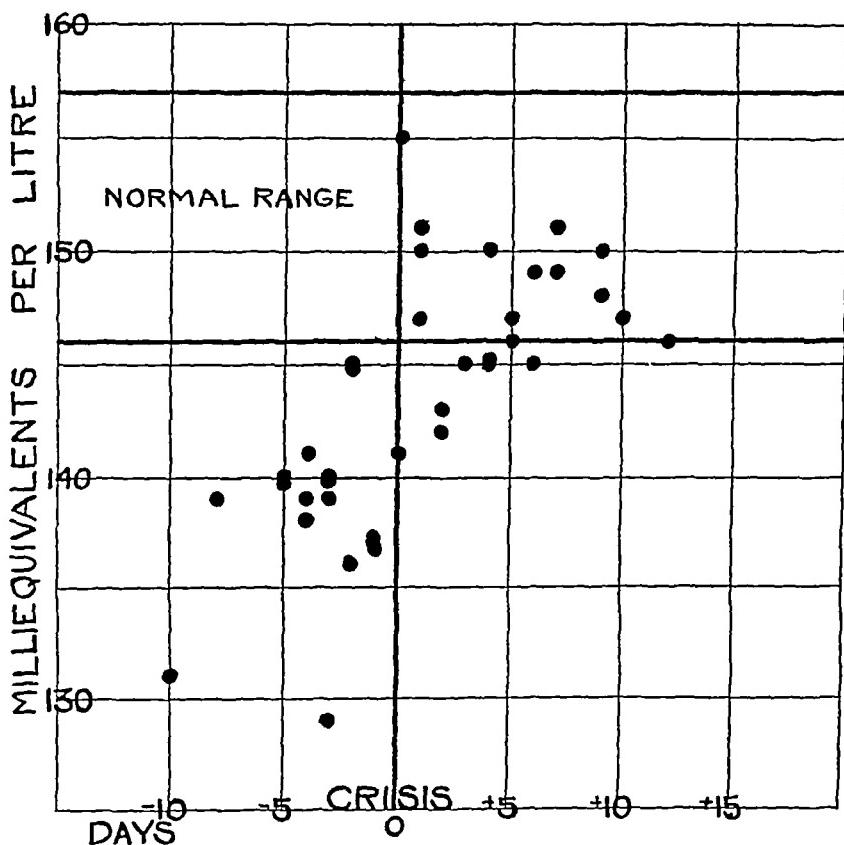


FIG 6 TOTAL BASE IN SERUM

determinations during the stage of active infection excepting in one case, designated G on the graph. In this case the blood urea nitrogen analyses on the various specimens were as follows

$G_1 = 110 \text{ mgm per } 100 \text{ cc blood}$

$G_2 = 111 \text{ mgm per } 100 \text{ cc blood}$

$G_3 = 41 \text{ mgm per } 100 \text{ cc blood}$

$G_4$  = not determined

$G_5$  = 15 mgm per 100 cc blood

While 110 mgm of urea nitrogen per 100 cc in  $G_1$  in which the freezing point depression was  $0.61^{\circ}\text{C}$  would only account for an approximate increase of  $0.02^{\circ}$  in the freezing point depression, the urea

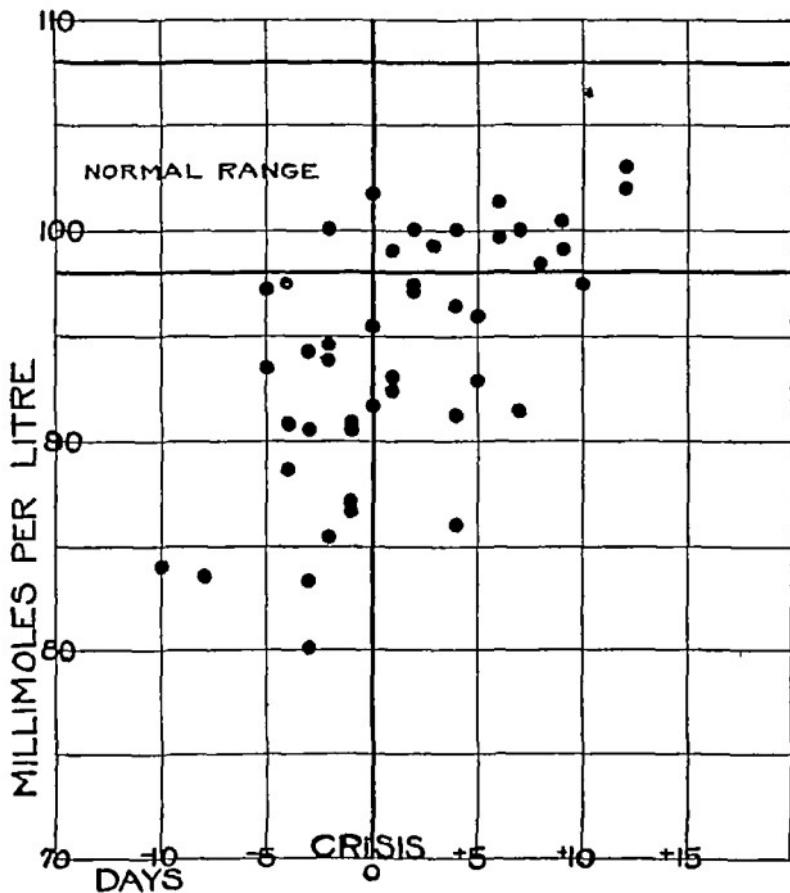


FIG 7 CHLORIDE IN SERUM

doubtless represents only a fraction of the non-electrolyte increase in this serum. With exception of this instance a diminished freezing point depression has consistently existed even in those observations made very early in the infection—e.g., the second day of disease. As

in the case of conductivity and base determinations the freezing point depressions of individual cases may show further diminution up to the ninth day. Immediately after the crisis the freezing point depressions for the most part were considerably higher than the

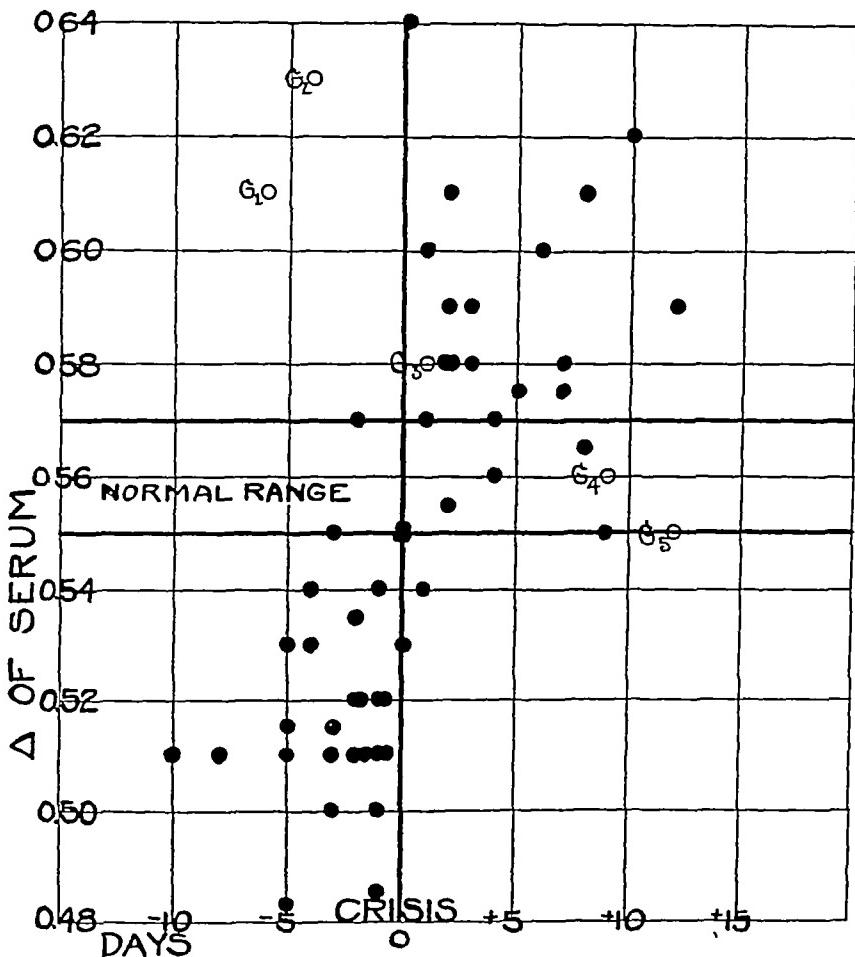


FIG 8 SERUM FREEZING POINT DEPRESSION ( $\Delta$  of serum)  
(G = case 16)

normal level. That this increase is not entirely due to an increased electrolyte content is obvious when the ratio  $\frac{\Delta}{C_e}$  is considered, where  $\Delta$  represents the freezing point depression and  $C_e$  the corrected conductivity in sodium chloride equivalents (fig. 9).

In normal individuals the ratio  $\frac{244\Delta}{C_s}$  varied from 0.98 to 1.03. This ratio remains constant when changes in the freezing point depression are due entirely to changes in the electrolyte content. Hence the ratio may serve as an index of the concentration of non-electrolytes in the serum. When  $\frac{244\Delta}{C_s}$  was calculated from our data, during

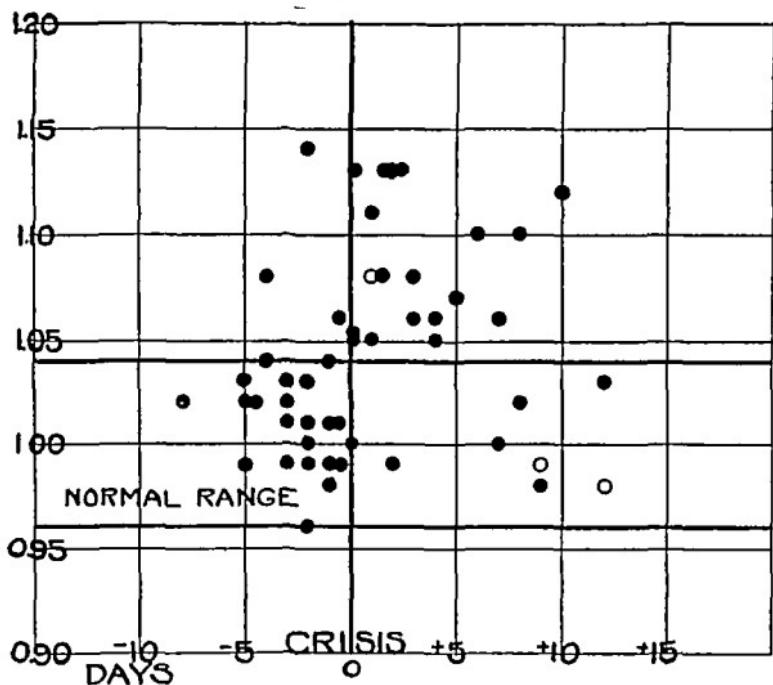


FIG 9 RATIO OF FREEZING POINT DEPRESSION TO CORRECTED CONDUCTIVITY  

$$\frac{\text{Freezing point depression}}{244 \frac{\Delta}{\text{Corrected conductivity}}}$$

$\circ$  refers to case "G" under freezing point depression or to case 16 on the tables

the active infection it was for the most part within the normal limits. After the crisis, however, the ratio was increased beyond the normal range especially during the first few days following the crisis, with the exception of one observation. Thus we conclude that the decreased depression in freezing point during the active infection can be accounted for by the decrease in electrolytes, whereas after the

crisis the increase in freezing point depression is caused in part by the presence of abnormally high concentrations of non-electrolytes

*Protein-corrected conductivity ratio* The refractometric protein measurements were made primarily to correct the conductivity values according to the formula of Gram and Cullen. However, with the data collected and several possible relations of the protein

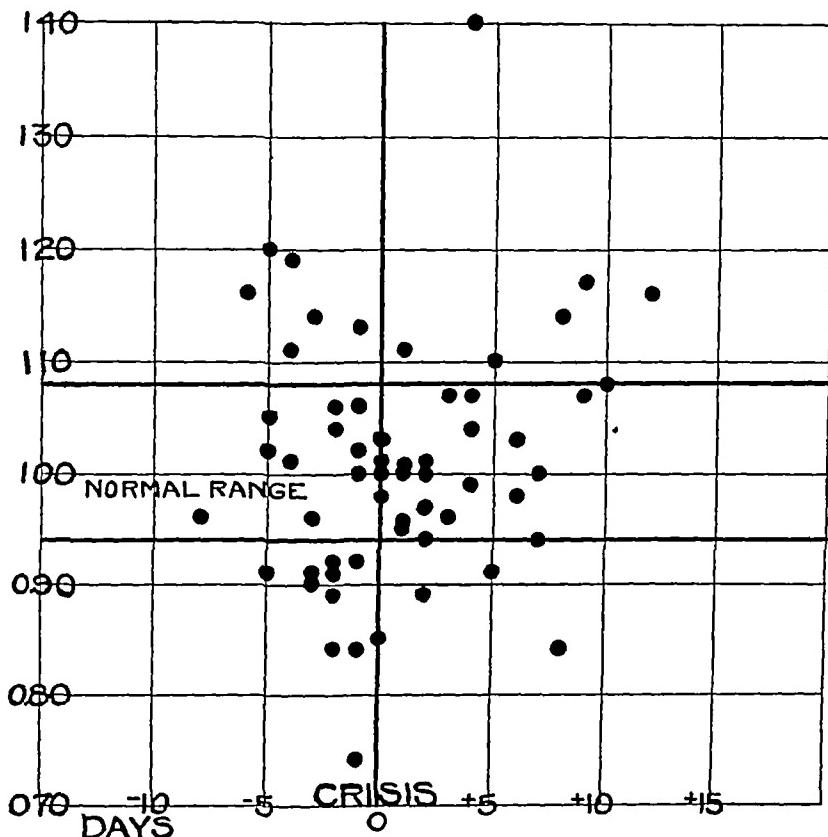


FIG 10 RATIO OF PROTEIN TO CORRECTED CONDUCTIVITY

$$\frac{16.9 \text{ Protein}}{\text{Corrected Conductivity}}$$

concentration to our results suggesting themselves, we regret that the protein determinations were not made by more accurate methods, thus avoiding the known discrepancies with refractometric measurements. While no conclusions are drawn from individual ratios of protein concentration and corrected conductivity equivalents, nevertheless, the trend of this ratio we are confident is significant.

It is shown on figures 1 and 4 that protein and corrected conductivity values tend to be lower during the precritical course and return to the normal range during convalescence. The percentage decrease in both approximates 10 per cent. Thus if the ratio of protein to corrected conductivity be considered, it is shown on figure 10 that the ratio fluctuates about an average value which remains approxi-

TABLE 1  
*Normal subjects*

Subject	Serum freezing point depression °C	Serum corrected conductivity mEq per liter	Serum protein (refractometric) gm per 100 cc	Serum total base mEq per liter	Serum chloride mEq per liter	$\frac{244}{C_c} \Delta$	$16.9 \frac{\text{Protein}}{C_c}$
F W S	0 555	136	7 7			1 00	0 96
C	0 55	134	7 7	152		1 00	0 97
R	0 56	133	8 3	154		1 03	1 05
P	0 57		8 1	150			
W	0 55		8 7	150		1 03	
Pe	0 555	131	7 6			1 03	0 98
S		134	7 8	157 6	103 7		0 98
Sel		136	8 4	151 7	101 2		1 04
Rei		137	8 0	154 8	105 3		0 98
Rob		134	8 6	153 4	103 5		1 08
C C S					105 8		
H W S					106 4		
C F					104 3		
H T					101 4		
Maximum	0 57	137	8 7	157 6	106 4		
Minimum	0 55	131	7 6	150	101 2		
Average	0 557	133	8 09	154 7	103 95		
Number of determinations	6	8	10	8	8		

mately the same throughout the entire infection. In normal individuals the ratio of  $16.9 \frac{\text{Protein}}{\text{Corrected Conductivity}}$  varied from 0.94 to 1.08. During the stage of active infection this ratio tends to fluctuate more than normally and perhaps more than after the crisis.

TABLE 2  
*Pneumonia*

Case number	Type of organism	Date	Day of disease	Day from crisis (crisis = 0 day)	Temperature	Pulse rate per minute	Respiration rate per minute	Sputum freezing point (°C)	Sputum corrected con. ductivity (C <sub>e</sub> )	2444 C <sub>e</sub>	Serum protein (relative to metric) (g/100 cc)	16.9 Protein g/100 cc	Serum dry weight per 100 cc	mEq per liter	mEq per 100 cc	Serum chloride per liter	mEq per liter	mEq per 100 cc	Blood urea nitrogen per 100 cc	Blood glucose per 100 cc	Blood creatinine per 100 cc		
					°F	°C	mm per 100 cc	mm per liter	mm per liter	mm per 100 cc	mm per 100 cc	mm per liter	mm per 100 cc	mm per liter	mm per 100 cc	mm per liter	mm per 100 cc	mm per liter	mm per 100 cc	mm per liter	mm per 100 cc		
1	Pneumococcus Type IV	January 5 January 19	2 16	-10 +4	103 4 97 8	96 24	0 51	130	10 8	1 40	131	84 0	24 7	8 3	91 2	140	93 4	22 9	15 2	140	83 2	25 7	20 1
2	Pneumococcus Type I	March 4 March 6 March 10 March 13 March 17	5 7 11 14 18	-5 -3 +1 +4 +8	105 0 104 4 97 8 97 0 97	96 100 80 72 72	0 53 0 50 0 60 0 57 0 57	125 1 123 0 132 1 131 1 135 1	8 9 8 3 8 7 8 3 9 1	1 20 1 14 1 11 1 06 1 02	10 0	140	147	99 0	145	100 0	28 6	17 9	1 3	145	100 3	25 6	20 1
3		February 18 February 19	3 4	-2 -1	102 2 101 0	92 96	22 22	0 52 0 52	128 0	0 99	8 0	1 06	1 06	1 06	98 3	150	99 0	28 9	15 5	123	1 01	6 8	0 92
4	Pneumococcus Type II	March 12 March 16 March 19 March 22	7 11 14 17	-1 +3 +6 +9	101 0 98 4 98 4 98 4	88 78 76 72	0 52 0 58 0 55 0 55	122 1 131 1 131 1 137 0	7 4 8 3 7 6 0 98	1 02 1 07 1 07 1 07	8 3 8 7 9 3 9 3	137	90 8	27 6	15 6	1 1	145	99 2	27 2	145	101 3	25 6	20 1
5		February 19 February 23	9 13	-5 -1	101 8 100 96	108 96	0 51 0 51	122 1 123 1	1 02 1 01	7 4 6 8	1 02 0 92	123	123	123	123	123	123	123	123	123	123	123	12 9

-6	Pneumococcus Type III	February 27	6	-4	102 0	104	36	0 54	122	1 08	8 6	1 19	9 4	139	97 4	20 5	22 1
7	Pneumococcus Type II	March 2	9	-1	101 6	96	28	0 49	120	0 98	8 0	1 13	9 1	137	86 6	24 4	14 5
8	Pneumococcus Type II	March 10	10	0	103 0	132	44	0 64	139	1 13	8 5	1 03	8 8	155	101 7	25 9	85 5
9	Pneumococcus Type II	March 15	12	+5	98 0	60	20		123	7 3	1 00			141	91 6	26 4	14 5
10	Pneumococcus Type II	March 17	14	-8	100 0	108	26	0 51	122	1 02	6 9	0 96	8 0	139	83 5	30 4	29 0
11	Pneumococcus Type IV	March 23	20	-2	99 2	92	24	0 51	129	0 96	7 0	0 92	7 9	145	100 0	27 2	12 5
12	Pneumococcus Type IV	March 27	24	+2	98 0	80	24	0 56	131	0 99	7 7	1 00		100	0	27 2	1 1
13	Pneumococcus Type I	March 29	26	+4	98 0	72	20	0 56	130	1 05	8 1	1 04	9 3	150	96 3	31 5	
		April 1	29	+7	98 0	72	16	0 58	141	1 00	8 3	1 00	8 7	151	91 3	31 1	
		March 3	11	+1	98 0	108	32	0 54	125	1 05	7 0	0 95	8 1	151	93 0	32 7	26 0
		March 11															1 3
		March 15	7	-2	102 6	88	40	0 52	127	1 00	6 3	0 84					
		March 16	8	-1	101 0	72	28	0 51	123	1 01	5 4	0 74					
		March 17	9	0	97 6	72	28	0 53	130	1 00	6 5	0 85					
		March 19	11	+2	96 6	60	20	0 58	125	1 13	6 6	0 89	8 0	143	97 3	27 6	
		February 1	5	0	97 4	84	24	0 55	128	1 05	7 7	1 01		95	4	25	1
		February 2	6	+1	97 8	80	20	0 59	139	1 06	7 8	0 95					
		February 4	8	+3	97 6	72	20	0 59	135	1 06	7 7	0 96					
		February 23	11	-2	104 8	120	44	0 57	122	1 14	7 5	1 04	8 3	136	94 5	25 8	21 5
		February 25	13	0	99 0	108	20	0 55	128	1 05	7 4	0 98					14 1
		February 27	15	+2	98 6	84	20	0 58	133	1 06	7 6	0 97					
		March 3	19	+6	98 2	88	20	0 60	133	1 10	8 1	1 03		149	99 6	25 3	14 6

TABLE 2—Concluded

Type of organism	Date	Case number	Day of disease	Day from crisis (days)	Pulse rate per minute	Respiration rate per minute	Serum freezing point depression (A°)	Serum corrected carbon dioxide capacity (C <sub>CO<sub>2</sub></sub> )	Serum protein (refractometric) (g Eq per 100 cc)	Serum dry weight	Serum total base	Serum chloride	Serum CO <sub>2</sub>	Blood urea nitrogen	Blood creatinine	
Pneumococcus Type II	February 17	18	+8	97.8	64	24	0.61	135	1.10	6.7	0.84	8.2	139	94.2	22.5	33.0
Pneumococcus Type II	January 21	22	+13	98.2	80	22	0.59	140	1.03	7.5	102.0	28.4	14.1	1.1	1.1	1.1
Pneumococcus Type II	February 23	7	-6	101.4	92	32	0.61	121	1.23	8.3	1.16	138	88.6	21.8	110.0	110.0
Pneumococcus Type II	February 25	9	-4	102.0	84	24	0.63	125	1.23	8.2	1.11	9.5	150	92.3	24.3	41.0
Pneumococcus Type II	March 2	14	+1	99.4	96	36	0.58	131	1.08	7.7	1.00	148	100.3	29.4	1.1	1.1
Pneumococcus Type II	March 10	22	+9	98.0	84	24	0.56	138	0.99	9.7	1.17	10.4	146	103.0	26.4	15.0
Pneumococcus Type II	March 13	25	+12	98.0	96	20	0.55	137	0.98	9.4	1.16	11.1	147	97.4	28.8	13.9
Pneumococcus Type II	February 23	5	-5	102.0	96	28	0.48	118	0.99	7.3	1.05	1.05	145	98.7	26.3	15.2
Pneumococcus Type II	February 25	7	-3	102.4	96	36	0.51	120	1.03	6.8	0.90	1.05	147	97.4	28.8	13.9
Pneumococcus Type II	March 1	11	+1	98.0	96	24	0.57	129	1.08	7.6	1.00	1.00	150	92.3	24.3	41.0
Pneumococcus Type II	March 10	20	+10	98.0	84	20	0.62	134	1.12	8.6	1.08	8.8	147	97.4	28.8	13.9
Pneumococcus Type IV	March 2	6	-2	101.0	116	32	0.535	127	1.03	6.7	0.89	1.05	145	98.7	26.3	15.2
Pneumococcus Type IV	March 8	12	+4	98.0	56	24	0.535	130	1.06	7.56	0.99	8.8	145	86.0	27.3	12.9
Pneumococcus Type IV	March 11	15	+7	98.0	72	15	0.58	133	1.06	7.44	0.94	1.06	149	100.0	27.9	14.9

19	Friedlander and Pneumococcus Type IV	February 1	8	-1	100 0	108	32	0 54	124	1 06	7 3	1 00			87 1	27 2		
20	Pneumococcus Type I	March 16	5	-4	103 0	108	32	0 53	124	1 04	7 38	1 01	8 6	141	90 7	27 7	25 2	1 2
		March 18	7	-2	103 0	128	32	0 51	123	1 01	6 57	0 91	7 8		85 3	25 5	30 2	1 3
21	Pneumococcus Type I	March 18	9	-3	101 4	120	44		116		6 58	0 96		129	80 0			
22	Pneumococcus Type I	March 22	5	-5	104 4	120	44	0 515	123	1 02	6 63	0 91	8 1	140	97 1	24 5	16 1	1 2
		March 24	7	-3	103 6	108	52	0 515	123	1 02	6 57	0 91	7 5	140	90 5	23 2		
		March 26	9	-1	103 6	126	56	0 50	123	0 99	6 1	0 84	7 3	137	90 5	23 2	16 2	1 2
		March 29	12	+2	98 0	72	28	0 59	127	1 13	7 19	0 94		142	97 1	23 9		
		April 1	15	+5	98 0	72	20	0 575	132	1 07	7 15	0 91	9 0	146	92 8	25 9		

## DISCUSSION

In considering the fate of the electrolytes during the precritical course in pneumonia, the question is raised whether the decrease in electrolyte concentration in the serum is due to a process of simple dilution

It can occur that the total amount of serum protein remains constant while the water content of the serum changes. Under these conditions change in serum protein concentration is an index of gain or loss of serum water. Such gain or loss of water is often accompanied by an associated gain or loss of salts, this is not always the case, however. Hamilton, Barbour and Loomis (1925) have made use of the  $\frac{\text{protein}}{\text{salt}}$  ratio in their studies on heat regulation and water exchange and have found conditions in which this ratio is not significantly altered when the water content of the serum is increased. In our studies the protein concentration and the corrected conductivity both showed approximately the same percentage decrease during the precritical period with the maintenance of a constant mean  $\frac{\text{protein}}{\text{corrected conductivity}}$  ratio. This might suggest that the decrease in concentration of electrolytes and protein was caused by a process of simple dilution. That this increase cannot be explained as incident to mere water retention in the body tissues, as a whole, is obvious when the known degree of sodium chloride retention in pneumonia is considered.

Hutchison (1898) pointed out that in the precritical period of pneumonia a retention of as much as 12 grams of sodium chloride is not unusual. If it be supposed that this is retained in a concentration equal to that found in lymph and serum, there would be necessary a simultaneous retention of about 20 liters of water. It is clear that this is not the case especially when the weight during pneumonia is considered. Leyden as early as 1869 showed that there was a progressive loss of weight in the precritical period in pneumonia patients while still greater loss occurred with the crisis.

The decrease in electrolyte concentration in the serum cannot therefore be considered a part of simple dilution of the body tissues,

as a whole, with water, but it will be necessary to have studies upon blood volume in pneumonia to determine whether or not the fall in electrolyte and protein concentration in the serum is due to simple dilution of the serum with water, while the retained chloride and perhaps also water are accumulated in the other tissues of the body. Such blood volume studies we have now in progress.

In spite of the loss of weight in pneumonia there is evidence in the literature that the ratio of water to dry substance is increased in certain of the other tissues of the body as well as in the serum. Hutchinson (1898) observed a slight increase in the water content of muscle and Maver and Schwartz (1916) found in all of the pneumonia cases studied, moderate degrees of edema in the subcutaneous tissues by the use of the Schade elastometer. The latter observers also noted that the elasticity curve did not return to the normal immediately after the crisis as did the chloride concentration of the blood.

Furthermore, according to Hutchinson (1898), the sputum, exudates, muscles, and viscera are all richer in chloride to total dry substance than normally. Peabody (1913) confirmed the widespread distribution of retained chloride in pneumonia and since no particular storage place was found, he believed that the retained inorganic substances are "spread diffusely throughout the body."

It may be possible that retention of both water and chlorides occur in the general tissues of the body, while in the serum possibly water alone is retained.

The precise nature of the non-electrolyte increase following the crisis has not been definitely determined. Cohn (1922) in an analysis of the blood of fifty five patients with pneumonia found an increase in the non protein nitrogen in 87 per cent of his cases. The increased values appear to have had no relation to temperature changes. Killian (1922) found that the non-protein nitrogen was increased after the crisis. There was an increase after the crisis in two cases which Haden (1925) reported. The maximum increase in his cases, however, would only account for about one-fifteenth of the post critical increase in non-electrolytes which is indicated by our data. Our results in regard to the nature of the non-electrolyte increase are not sufficient to draw conclusions.

In the two cases in which the blood cholesterol was determined

before and after the crisis, there was a post-critical hypercholesterolemia and in this our results would confirm the work of Kipp (1920) (The cholesterol results are recorded in protocols 1 and 15)

#### SUMMARY

The percentage of protein in the serum as determined by means of the refractometer and Reiss' constant was approximately 90 per cent of the dry weight. The protein values tended to be below the normal during the febrile period and rise to the normal following the crisis.

CO<sub>2</sub> content remained approximately within the normal range throughout the disease.

The corrected conductivity was consistently below the normal during the precritical period and following the crisis rose to the normal limits.

The corrected conductivity obtained with the factor of Gram and Cullen and expressed in milliequivalents of NaCl was related to the total base within the limits of 3 per cent as follows  $1.13 C_e = \text{total base}$

The total base concentration in the serum was decreased during the active infection and tended to become normal after the crisis.

The chlorides were below normal during the precritical course and tended to reach the normal more slowly than the total electrolytes following the crisis.

The ratio  $\frac{\Delta}{C_e}$  is within the normal limits during the active infection and is increased especially during the first five days following the crisis.

During the active infection in lobar pneumonia there was a decrease in the electrolytes in the serum and a proportional decrease in the freezing point depression. After the crisis the electrolytes returned to the normal range, whereas the freezing point depression was increased beyond the normal limits. This increase after the crisis must therefore be due to abnormally high amounts of non-electrolytes in the serum.

The ratio of protein to corrected conductivity fluctuates about an average value which remains approximately the same throughout the entire infection.

## PROTOCOLS

*Case 1 (No 276)* Colored, male, age 37, laborer On admission, the second day of the disease, the patient had consolidation of the right lower lobe He appeared extremely toxic and was irrational most of the time during the active infection The blood culture was sterile sputum yielded a Type IV pneumococcus. The right upper lobe became involved on the tenth day of the infection The crisis occurred on the twelfth day Convalescence was uneventful Wasser mann was strongly positive before and after the crisis. The blood cholesterol on the second day of the disease was 123 mgm per 100 cc , on the sixteenth day, 192 mgm per 100 cc

*Case 2 (No 1220)* Colored, male age 19, laborer The patient was admitted to the hospital on the fourth day of the disease with consolidation of the left lower lobe. He was wildly delirious and required restraint. The leucocyte counts on the fourth and fifth days of the disease were 37,000 and 36,400 respectively, the polymorphonuclears being 94 per cent The blood culture was negative, sputum contained a Type I pneumococcus.

*Case 3 (No 1009)* Colored male, age 48, laborer The patient was admitted with incipient delirium tremens and lobar pneumonia confined to the right lower lobe. The temperature returned to the normal by crisis on the fifth day of the disease. Convalescence was uneventful.

*Case 4 (No 1365)* American, male, age 46, printer Five days before admission the patient noticed a dull pain in left lower chest, a brown tinged expectoration, and fever At the time of admission he appeared moderately toxic and cyanotic The sputum showed a Type II pneumococcus The leucocyte count was 32,200

*Case 5 (No 1015)* Italian, male, age 35, stone mason On admission the patient had a dusky cyanosis of the face, herpes on the lips and nose, and a low, muttering delirium The respirations were rapid (fifty four per minute), restricted and shallow The physical signs of consolidation were present over both the right and left lower lobes. The leucocytes were 10,200 on admission with 90 per cent polymorphonuclears. Four days later the leucocyte count was 24,600 The abdomen was greatly distended and polypnea was marked throughout the entire active infection

*Case 6 (No 1146)* Colored, male, age 18, student On admission the pneumonia was localized to the right lower lobe The blood culture was negative sputum yielded a Type III pneumococcus The leucocyte count was 26,400 with 87 per cent polymorphonuclears. The patient, in addition, had a mitral stenosis which had followed rheumatic fever in childhood. On the ninth day the patient had a pseudo-crisis which was followed on the tenth day by the true crisis.

*Case 7 (No 1287)* American, male, age 46, policeman The patient was admitted to the hospital on March the sixth with consolidation of entire right lung The leucocyte count was 17,800 with polymorphonuclears 86 per cent On March the eighth the blood urea nitrogen was 20.2 mgm and the creatinine 2.8 mgm per 100 cc The patient was extremely cyanotic, unconscious, and breathed rapidly with considerable effort The abdomen was greatly distended Phlebotomy was done on March the ninth and 820 cc of blood were removed The patient was given hypodermoclysis of normal salt solution in 1000 cc quantities on two occasions on March 10 The patient died on March 10 at a time which was interpreted as being the crisis

*Case 8 (No 1274)* Hebrew, male, age 10, student Consolidation of the lung was limited to the right upper lobe The leucocyte count on admission was 30,000 Crisis occurred on the seventh day Convalescence was uneventful

*Case 9 (No 1370)* German, male, age 26, laborer The patient was admitted to the ward with consolidation of the right lower lobe On the following day, the eighth day of the disease, it was noted that the left lower lobe also was involved The sputum contained a Type II pneumococcus During the period of active infection the patient was wildly delirious The urine showed a heavy cloud of albumen, granular casts, and red blood cells On two occasions sugar was present in the urine (March 10—0.35 per cent) The temperature reached normal by lysis on the twenty-second day The Wassermann reaction was very strongly positive before and after the temperature became normal

*Case 10 (No 1172)* Italian, male, age 44, laborer The patient entered the hospital with a right lower lobar pneumonia The sputum yielded a Type II pneumococcus The leucocytes on admission were 31,000 with 92 per cent polymorphonuclears The crisis occurred on the tenth day On the fifteenth day the patient developed a fever and had a leucocyte count of 21,600 Physical signs of a right interlobar empyema were found and the patient was transferred to the surgical service for thoracotomy

*Case 11 (No 879)* American, male, age 25, laborer On admission the patient suffered from excruciating pains in left lower chest over which area pleural frictions were heard The chest was strapped but without much relief Blood culture was sterile the sputum yielded a Type IV pneumococcus

*Case 12 (No 702)* White, male, age 39, laborer The patient was admitted to the hospital on the third day of the disease with consolidation of right lower lobe The sputum yielded a Type IV pneumococcus and a hemolytic streptococcus The crisis occurred on the fifth day

*Case 13 (No 1076)* Colored, male, age 39, laborer On admission, the tenth day of the disease, the patient had pneumonia of the right lower lobe. The leucocyte count was 19,000 which rose to 30,200 the following day with 92 per cent polymorphonuclears. The patient appeared very toxic and was moderately jaundiced The blood culture and sputum both yielded a Type I pneumococcus. The urine contained a heavy cloud of albumen with many dark granular casts The crisis occurred on the thirteenth day Convalescence was uneventful.

*Case 14 (No 789)* Italian male age 36, laborer The pneumonia was confined to the right lower lobe The patient was delirious, cyanotic and appeared extremely toxic. The leucocyte counts ranged between 16,000 and 20,600 during the precritical course. Following the crisis, on the tenth day of the disease, the patient developed a gluteal pneumococcus abscess which was incised and drained

*Case 15 (No 252)* Italian, male, age 15, student. The patient was admitted to the hospital on the third day of the disease with a consolidation of the left lower lobe. The respirations were very rapid (60 per minute), and voluntarily restricted due to intense pleural pain The sputum contained a Type II pneumococcus The leucocyte count was 17,800 with 84 per cent polymorphonuclears. The crisis occurred on the ninth day The blood cholesterol on the sixth day of the disease was 137 mgm per 100 cc , on the twenty second day, 173 mgm per 100 cc

*Case 16 (No 1095)* Colored, male, age 35, laborer On admission the sixth day of the disease, the patient had a consolidation of the right upper and middle lobes. The leucocyte count was 26,800 with 95 per cent polymorphonuclears. Three days later, the ninth day, the right lower lobe became involved at which time his leucocyte count was 26,600 The patient was very weak and markedly jaundiced The sputum contained a Type II pneumococcus On the twelfth day of the disease there was an attempted crisis, however, the true crisis did not occur until the thirteenth day The urine contained a trace of albumin with occasional light granular casts. The blood urea nitrogen was greatly increased during the precritical period and returned to the normal during convalescence. The Wassermann reaction was strongly positive before and after the crisis.

*Case 17 (No 1060)* Egyptian, male, 31, painter The patient had a pneumonia of the right lower lobe. On admission the leucocyte count was 8,200 which rose three days later to 21,400 The patient was mentally below the normal. He gave a history of previous antiluetic treatment and his Wassermann reaction before and after the crisis was very strongly positive. The crisis occurred on the tenth day

*Case 18 (No 1162)* Colored, male, age 23, laborer The consolidation was limited to the left lower lobe. The leucocyte count was 30,500 with 95 per cent

polymorphonuclears. Sputum contained a Type IV pneumococcus. The crisis occurred on the eighth day of the disease. In addition to the pneumonia, the patient had a luetic aortitis and an acute arthritis. Wassermann reaction was very strongly positive.

*Case 19 (No 689)* American, male, age 38, laborer. The patient entered the hospital with a lobar pneumonia of the left base and an acute pleurisy. He appeared toxic and cyanotic. The sputum contained a Friedlander's bacillus and a Type IV pneumococcus. The temperature reached the normal level on the ninth day. On the eleventh day the temperature was elevated to 100°F and the leucocyte count was 19,400. At this time, physical signs of an interlobar empyema were obtained. A thoractomy was done and the pus drained. The patient died on the thirty-sixth day of the disease.

At autopsy both lungs were riddled with recent multiple abscesses. A dark field examination of the abscess walls proved the tissue to be literally alive with spirochetes of the Vincent type and fusiform bacilli.

*Case 20 (No 1413)* Colored, male, age 34, laborer. The patient had a left lower lobar pneumonia. The blood culture and sputum both yielded Type I pneumococcus. On two occasions the patient was given 100 cc of Type I anti-pneumococcus serum intravenously. Death occurred on the ninth day of the disease, at a time interpreted as the crisis. The diagnosis was confirmed at autopsy.

*Case 21 (No 1400)* Colored, female, age 30, housewife. On admission the third day of the disease the patient had consolidation of the right lower and middle lobes. The leucocyte count was 40,200 at this time. On the ninth day of the disease the left lower lobe was involved and the leucocyte count rose to 48,800. The sputum contained a Type I pneumococcus. Crisis occurred on the twelfth day.

*Case 22* Spaniard, age 24, sailor. The patient was admitted on the fifth day of the disease with consolidation of left lower lobe. His respirations were very rapid and voluntarily restricted on account of intense pleural pains. Loud pleural frictions were heard in left axilla. The leucocyte count was 23,200 with the polymorphonuclears 90 per cent. The sputum yielded a Type I pneumococcus, blood culture was sterile. Crisis occurred on the tenth day.

#### BIBLIOGRAPHY

- Austin, J H, and Gram, H C *Jour Biol. Chem.*, 1924, lix, 535 The Effect of Ether Added in Vitro on the Carbon Dioxide and Chloride Distribution between Cells and Serum  
Burian, R, and Drucker, K *Centr Physiol.*, 1909-10, xxiii, 772 Gefrierpunktgemessungen an kleinen Flüssigkeitsmengen

- Christiansen, J Wien Klin Woch , 1922, xxxv, 461 Eine einfache klinische Methode zur Leitfähigkeitbestimmung
- Cohn, E Deut. Med Woch 1922 xlvi, 226 Ueber das Verhalten des Rest stickstoffs im Blute bei kruposer Pneumonie, Scharlach, Masern und Diphtherie.
- Domarus A Methodik der Blutuntersuchung mit einem Anhang, Zytodiagnostische Technik. Berlin, 1921, p 211
- Folin, O Lab Manual of Biological Chemistry 1922 (Appleton)
- Gerstenberger, H J, Burhans, C W, Smith, D N, and Wetzel, N C Amer Jour Dis Child , 1923, xxvi, 329 The Blood Serum Content of Inorganic Phosphorus and Calcium in Pneumonia.
- Gram, H C Jour Biol Chem , 1923, lvi, 593 Observations on Regulation of Osmotic Pressure (Conductivity, Chlorides, Freezing Point, and Proteins of Serum)
- Gram, H C Amer Jour Med Sci , 1924, clxviii, 511 Composition and Physical Properties of Normal Human Blood, A Compilation of Values from the Literature.
- Gram, H C , and Cullen G E Jour Biol Chem , 1923, lvi, 477 The Accuracy of the "Ionometric" Method and of the Protein Correction in Measuring Serum Conductivity
- Haden, R L Jour Lab and Clin Med., 1925, x, 337 The Relation of the Chloride Metabolism to the Toxemia of Lobar Pneumonia.
- Hamilton, W F, Barbour, H. G , and Loomis, N M Jour Physiol , 1925, lix, 306 Heat Regulation and Water Exchange. VI The Mobilization of Water and Salt in Warm Environments.
- Hastings, A. B , Neill, J M Morgan, H J and Binger, C A L Jour Clin Invest , 1924, i, 25 Blood Reaction and Blood Gases in Pneumonia.
- Hutchison, R. Jour Path and Bact , 1898, v, 406 Chloride Metabolism in Pneumonia and Acute Fevers.
- Jansen, W H Deut Arch fur Klin Med , 1924, cxliv, 14 Kalkstudien am Menschen Der Kalkgehalt des menschlichen Blutes unter pathologischen Verhältnissen
- Kilban, J A Proc. N Y Path Soc , New Series 1921-1922, xxii, 72 Chemical Changes in the Blood in Pneumonia.
- Kipp, H A Jour Biol. Chem , 1920, xliv, 215 Variation in Cholesterol Content of the Serum in Pneumonia
- Leyden, E Arch fur Klin Med , 1869, v, 273 Untersuchungen über das Fieber
- Maver, M E , and Schwartz, A. B Arch Int. Med , 1916, xvii, 459 Studies of Edema in Pneumonia
- McLean, F C Jour Exp Med., 1915, xxii, 366 The Numerical Laws Governing the Rate of Excretion of Urea and Chlorides in Man
- Meakins, J Jour Path and Bact., 1921, xxiv, 79 Observations on the Gases

- in Human Arterial Blood in Certain Pathological Pulmonary Conditions, and Their Treatment with Oxygen
- Myers, Victor C Practical Chemical Analysis of Blood 1924, Mosby
- Peabody, F W Jour Exp Med , 1912, xvi, 701 The Carbon Dioxide Content of the Blood in Pneumonia
- Peabody, F W Jour Exp Med , 1913, xvii, 71 Studies of the Inorganic Metabolism in Pneumonia with Especial Reference to Calcium and Magnesium
- Peters, J P , Bulger, H A , Eisenman, A J and Lee, C Jour Biol Chem , 1926a, lxvii, 175 Total Acid-Base Equilibrium of Plasma in Health and Disease IV The Effects of Stasis, Exercise, Hyperpnea, and Anoxemia, and the Causes of Tetany
- Peters, J P , Bulger, H A , Eisenman, A J , and Lee, C Jour Biol Chem , 1926b, lxvii, 219 Total Acid-Base Equilibrium of Plasma in Health and Disease V Miscellaneous Pathologic Conditions
- Schade, H Zeitsch f exper Path u Therap , 1912, xi, 369 Untersuchungen zur Organfunction des Bindegewebes
- Stadie, W C Jour Exp Med , 1919, xxx, 215 The Oxygen of the Arterial and Venous Blood in Pneumonia and Its Relation to Cyanosis.
- Stadie, W C , and Van Slyke, D D Jour Biol Chem , 1920, xli, 191 Studies of Acidosis XV Carbon Dioxide Content and Capacity in Arterial and Venous Blood Plasma
- Stadie, W C , and Ross, E C Jour Biol Chem , 1925, lxxv, 735 A Micro Method for the Determination of Base in Blood and Serum and Other Biological Materials
- Van Slyke, D D and Stadie, W C Jour Biol Chem , 1921, xlvi, 1 The Determination of the Gases of the Blood
- Van Slyke, D D Jour Biol Chem , 1923, lviii, 523 The Determination of Chlorides in Blood and Tissues

# AN EXPERIMENTAL STUDY OF CHRONIC AORTIC REGURGITATION IN DOGS

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## INTRODUCTION

The present experiments were undertaken in an attempt to determine the progress of heart dilatation or hypertrophy, when the aortic cusps had been damaged, under conditions where there was no infection and where myocarditis could therefore be excluded. In some animals of our series infection occurred accidentally, but as a general rule there was no evidence of such a complication. Before and after operation the condition of the animal was determined by x-ray and electrocardiographic examination, and in addition, in some animals, tests were made to determine the capacity for work. The animals were kept for periods varying from 2 to 11 months.

No new principle was introduced in the above experiments, which differed from those of earlier workers merely in details, and in the use of modern methods. Cohnheim (1) first in 1877 described acute experiments of this type in dogs, perforating the aortic cusps by passing a button-shaped metallic sound down the carotid artery, finding he could destroy 1, 2, or even 3 valves. He noted that in such experiments a murmur, usually whistling in type, was at once heard, the pulse became water hammer in type, the heart compensated for the lesion and was able to maintain normal mean arterial and venous pressures, unless the lesion was too severe, when complete failure and death supervened. He was unable to produce an intermediate degree of damage, with slowly developing heart failure. Rosenbach (2) in 1878 working under Cohnheim's direction elaborated these results and made chronic experiments. Marey (3) in 1881 performed similar operations in horses and demonstrated an abnor-

mal rise of intraventricular pressure during diastole, the kymograph tracings he reproduced also demonstrated an increase in pulse rate of almost 100 per cent as the result of the lesion Tangl (4) in 1889 used a similar technic in chronic experiments to investigate heart hypertrophy and concluded that the changes depended on the time and degree of leak and that the number of fibers in such a heart were not increased, but that each individual fiber was larger

Hasenfeld and Romberg (5) in 1897 repeated such experiments aseptically in both cats and dogs They observed hypertrophy of the left ventricle under these conditions in the absence of sepsis, basing their conclusions on the weights of the different chambers of the heart and on the microscopic diameters of the fibers as compared with the normal They noted that hypertrophy was present in the left ventricle with some dilatation even with small lesions With larger leaks these changes were greater and also affected, but to a less extent, the left auricle and right ventricle They did not explain their evidence for dilatation, but probably they meant an increased heart cavity They noted also the complete absence of symptoms in the animals unless the lesions were great, when again sudden death was observed They considered that the hypertrophy was due to the increased work and that it was definitely compensatory, since after its development the animals were able to make a rather better response to experimental aortic constriction than in acute experiments In their chronic experiments inelasticity and tortuosity of the arteries developed No striking change of pulse rate was observed

H A Stewart (6) in 1908 introduced the theory that the collapsing pulse of aortic insufficiency depended on peripheral vascular dilatation, rather than on a regurgitation of fluid He found, however, intraventricular diastolic pressures as high as 40 mm of Hg and advanced the hypothesis of a regurgitation of pressure with minimal fluid reflux His evidence for this rested on heart plethysmographic records following regurgitation, since no increased difference between systolic and diastolic volumes was observed, as might have been expected if there had been much pendulum blood his conclusions, however, were only warranted if the filling of the right ventricle remained unchanged, and of this he had no evidence In a later

paper (7) he examined the effect of chronic lesions on heart hypertrophy and concluded that changes in heart weight began to take place within 5 days. His evidence was poor since it depended entirely on average heart weight to body weight ratios, and his normal animals from which the weight standard was set included dogs as large as 21.85 kilos, while his experimental animals were never above 8.1 kilos in weight. Since it has been shown by G N Stewart (8) that the heart weight is not a simple function of body weight in dogs, the sets of figures obtained by H A Stewart were not comparable, and such exact deductions as to the day hypertrophy commenced were inadmissible.

MacCallum (9) described in 1906 a special valvulotome (used by H A. Stewart) and himself made experiments of this type in 1911. He criticised Stewart's hypothesis, and showed a great fall of diastolic pressure in the aorta even when the heart was connected to a reservoir offering a constant peripheral resistance, and where vascular dilatation could not play a part.

Wiggers (10) in 1915 in acute experiments recorded the arterial and ventricular pressure changes photographically, concluding that the fall of aortic pressure during diastole was steeper when the aortic leak was greater, and that the rapid fall of pressure depended therefore on the condition of the valves rather than on peripheral dilatation. In his book on the circulation Wiggers (11) has taken the position that a considerable "regurgitation" of pressure may occur with only minimal back flow of fluid.

Sherrington (12) pointed out that the fall of diastolic pressure with regurgitation is as readily seen in the spinal animal, where all the vessels are already fully dilated, as in the intact animal.

Straub (13) in 1919 discusses the mechanisms of adjustment to aortic regurgitation and gives experimental data from plethysmographic records but without details.

Preliminary reports of our own (14) (15) work have appeared already. Still more recently Herrmann (16) has reported data in dogs kept 2 to 530 days after production of such lesions, where electrocardiographic records were taken, but where heart hypertrophy was estimated only by the relative weights of the two ventricles in comparison with body weight and normal standards. Any definite

changes have lowered the resistance to infection in the heart and the ligation of both carotids has done the same for the wound. Consequently the mortality of the second operation, was much higher than that of the first. The production of lesions of three valves in a single operation was immediately fatal, with one exception (see experiment C). On the other hand by operating in two stages, big lesions of two or three valves were successfully produced in five animals with the production of no marked disability, except in two after the development of infection. Occasionally in the single operation extensive lesions of only two valve cusps were sufficient to cause immediate death.

Since in our experiments large changes in pulse rate have occurred, attempts have been made to exclude this factor as far as possible either by section of both vagi or by atropine injections. The former of these procedures proved disappointing. In one such experiment (experiment G) the right vagus was divided just distal to the origin of the recurrent laryngeal within the chest (an operation very kindly performed for us by Dr Sweet) the left vagus was later divided in the neck and still later regurgitation produced. The autopsy, however, showed an aberrant branch of the right vagus arising above the recurrent laryngeal and passing to the heart. Since recovery from this operation was also complicated in this animal by persistent vomiting, recourse was had to atropine injections and in many instances the actual lesion was itself produced under the effects of atropine.

To test the ability of the animal to do work, and at the same time to increase the probability of the development of a real cardiach hypertrophy, some of the dogs were made to exercise on a treadmill. This was built of the same general type as that described by Kulbs (20). The treadmill was set at an angle varying between  $10^{\circ}$  and  $12.5^{\circ}$  and the animals were able to control the amount of work performed. They were tied on the mill for one hour or less, and were left to do what work they chose. The dogs soon learned to steady the machine by leaning against the side, so that they could rest or lie down as desired. Exercise was usually given four or five times a week, and note taken of the distance voluntarily travelled. The climb was calculated from the angle of the tread, and represents approximately the relative amount of work done, since when travelling fast the animal

maintained its position on the inclined plane by its own movements. No accurate estimation is, however, possible, since at times the animal supported a considerable proportion of its weight by dragging on its collar.

The surviving animals were ultimately killed, generally by chloroform. In a few cases, blood pressures were taken at this time using a Hürthle manometer connected alternately with brachial and femoral arteries in the same way as already described for acute experiments (21).

Immediately after death the heart was removed, drained of blood and weighed, one large cannula was then tied into the aortic arch just above the valves, and a second into the left ventricle, and the volume per minute leaking back into the ventricle from the aorta was then measured, using approximately a pressure of 1 meter of saline (73 mm of Hg).

In some of the later experiments, after removal of the cannulae and stitching of the ventricular wall, casts were made of the cavities of the right and left ventricles. This was done by the simple method of using a paraffin vaseline mixture setting at about 45°C, and pouring it into the two ventricular cavities through a short funnel, the heart being held up by the auricles until the two masses had set. Though the cavities are in this way distended at very low pressures, and the actual volumes may be very different from those existing in life, the casts obtained allow a good comparison of the *relative volumes of the two sides*. Any such comparison is quite valueless if there is some rigor mortis of the heart, since this rarely comes on at the same time in the two sides, immediately after death this error does not arise. In one animal death occurred unexpectedly and the heart was in rigor at autopsy. In this case casts were made after tap water had been perfused through the coronary arteries (with massage) which removes the rigor leaving what appears to be a uniform water turgor. A few control experiments on hearts of normal dogs in rigor mortis indicated that if this procedure were followed comparable casts were obtained from the two ventricles, though no doubt the absolute volume had been altered. Keller (22) has found that, if rigor can be excluded, the casts of the two sides are of equal volume, but his figures demonstrate the immense errors present as soon as rigor mor-

tis occurs If proper precautions are taken these errors should be rare The following table contains figures obtained on four hearts soon after death in normal dogs

Weight of dog kgm.	Weight of heart grams	Right ventricle volume cc.	Left ventricle volume cc
8 5	60 5	10 0	9 7
	71 0	4 5	5 2
9 25	75 0	27 5	9 7
13 0	106 0	22 8	23 7

The figures demonstrate only slight variations between the two sides except in the third where commencing rigor was suspected Three other hearts in full but irregular rigor were perfused with water and gave volumes for the left ventricle in comparison with the right of 100, 107 and 97 per cent respectively, showing a remarkably consistent equality We consider therefore that the method gives a comparison of the two ventricles which will only rarely be deceptive After a rough macroscopic examination had been made of the heart, it was fixed in formalin and sent to Dr R T Grant of University College, London, who is investigating this material in more detail, and will report his findings independently

In addition we are indebted to Dr B E Lucké for making some other histological and bacteriological examinations

#### EXPERIMENTS

While 23 dogs have been used in the course of this research, yet two of these were used as controls only and two others died of trauma at the first operation Of the remaining 19, 2 were killed within 1 to 3 days of the operation in order to determine the early condition, and 11 survived the operation until they were either killed or were operated on a second time Seven dogs in all had a second operation and of these 2 died on the table from trauma to the aortic arch, 2 died a few days later from septicemia, and 3 survived for months Two other animals survived the initial operation for a few days, but only one of these appeared to have real decompensation, and it was the only animal in the whole series, that clinically showed any signs

of progressive failure These 2 animals will be considered later (experiments C and H) Four animals died on the table at the first operation as the result of the lesion causing too great a regurgitation, or interfering with the coronary circulation

*Experiment A—Dog 5*

Male, 10 kilos Normal electrographic and heart sound records and short exposure x rays with tube to film distance of 80 cm were taken before operation, all under morphine The dates when records were taken can be seen in table 1

In this and the succeeding tables the symbols used have the following significance

PP = duration of the P wave.

PQ = start of P to beginning of Q or R

QS = duration of QRS group

QT = start of Q to end of T

Mechanical systole = interval between the beginning of the first and second heart sounds

R to second = peak of the R wave to the beginning of the second sound

T to second = end of the T wave to the beginning of the second sound Where the second sound begins before the end of T these values are negative and are italicized in the table

K, K', M and M' are factors by which the square root of the cycle must be multiplied to obtain QT ST, first to second sound and peak of R wave to second sound Any considerable variations in these values indicate a change in the duration of systole not explicable simply on the basis of variations in pulse rate

The area given for the x ray is measured without correction for tube distance and is expressed in square centimeters If the exposure has been instantaneous, the columns indicate the relation of the exposure to respiration and to the heart cycle In the latter case the time is given in 1/10 seconds between the beginning of the exposure and the previous wave of the E C G and between the end of the exposure and the next E C G wave, comparison being made to the peak of P, R S and the end of T The pulse rate figures express the average of 4 or 5 cycles previous to the exposure

January 22, 1923 2 15 p.m., morphine sulphate, 50 mgm 2 45 p.m., records of E C G and sounds. 2 55 p.m., chloroform-ether then pure ether One cusp ruptured with immediate increase in pulse rate. Fluoroscopic examination showed slight decrease (1 mm) in the transverse diameter, and no recognizable change in the longitudinal axis. 3 20 p.m., operation finished—ether discontinued 3 30 p.m. E C G and sounds About 3 40 p.m., 2 x rays with E C G checks but animal restless, coming out of ether 4 00 p.m. dog running about room—staggering, but no circulatory deficiency noticed

January 23 1923 The dog perfectly fit good appetite, running and jumping,

TABLE I  
Experiment 4—Dog 3



or standing on hind legs, but not quite so excitable as usual No dyspnea Extra systoles, probably left ventricular, occasionally present

January 25, 1923 Exercised in yard for 10 minutes just before records taken

January 26, 1923 2 15 p m, morphine sulphate, 100 mgm 3 37 p m, chloroform 3 51 p m, blood pressure record from femoral on Hurthle manometer Pulse rate 89 Pressure 275/75 3 58 p m, x-rays and E C G records until death The changes preceding death have been already given in a previous paper (tables 3 and 7) Sample x-ray photographs are reproduced in figure 1



Fig 1a



Fig 1b

#### FIG 1 EXPERIMENT A

a Before operation, fourth x-ray of January 18, (see table 1)

b Three days after operation, third x-ray of January 25, table 1

Below each x-ray is mounted the electrocardiogram taken at the same time, the time of exposure being indicated by the induction currents on the electrical record Below the electrocardiogram is the respiratory record, the up stroke indicating expiration Time intervals  $\frac{1}{10}$  second

*Autopsy* Heart very distended—pericardium tense With 1 meter pressure of saline in aorta 2 3 liters per minute leaked into left ventricle A hole was present in the posterior cusp (about 7 mm by 3 mm) close to its attachment The margins were thickened and rounded There was also some thickening of the endothelium on the interventricular septum and on the aortic cusp of the mitral valve Weight of heart 91 grams, i e , 0 91 per cent of body weight (The normal ratio according to Herrmann is 0 798 per cent for dogs of varying weights and according to Joseph 0 720 for male dogs of this weight) The left ventricular wall appeared to be slightly thickened, but the change was not definite Microscopic examination by Dr Grant showed the thickening of the edges of the lesion to be due to fibrosis with no indication of active inflammation We observed

absolutely no disability from such a lesion of a single cusp, which however gave a very definite water hammer pulse and big pulse pressure, though the pulse rate increase was only moderate. It was also noticeable that immediately after operation and on the following day the heart shadow area was always smaller than in any of the control photographs, while six weeks later shadow areas larger than any of the control values were obtained.

#### *Experiment B—Dog 15*

Male Initial weight 100, final 122.5 kilos. Increased weight due to fat. On each day electrocardiograph and heart sound records were taken without morphine but 40 to 150 mgm. of morphine sulphate were given before long composite x ray exposures in the expiratory position were taken (tube to film distance 1 meter). Dates of records and of operations are indicated in table 2 (see also figs 2 3 4). Exercise on treadmill for 30 to 45 minutes on May 7 and 8, but only travelled 1 kilometer in this time, became short of breath, took frequent rests and vomited afterwards. Exercise tried again on 4 days between May 12 and 18 with similar results. Average work 1.13 kilometer in 37 minutes with "climb" of 194 meters. July 12 to September 9, 1924, exercise repeated during periods separated by a three weeks rest, leaving a total of 28 days work during the whole period. Average distance travelled was 1.45 kilometer with a climb of 253 meters. The animal worked on the average for 49 minutes, but rested for about 19 minutes of this time. Leaving him on the treadmill for 60 to 70 minutes did not increase the work done as after the first 15 minutes he seemed fatigued and could not be coaxed to do much more work. Occasionally after work especially in warm weather the dog vomited. During some cold days in the middle of this period the amount of work done was much greater, reaching on one day 4.78 Kilometers. Work was done on 14 days during the last month of life with an average distance travelled of 1.09 kilometers and a climb of 190 meters only. The decreased work was coincident with an increased room temperature. Though the power to do work was thus limited, the animal did not appear in any way ill and was as lively as any normal dog.

December 17 1925 Morphine, 150 mgm Chloroform followed by ether. Right vagus dissected stimulated and no evidence of regeneration found. Blood pressure by Hürthle manometer Brachial 121/52 and femoral 137/47, pulse rate 106. Killed with chloroform.

*Autopsy* Pericardium contained 1 or 2 cc. of fluid. With 1 meter of water pressure 2.8 liters regurgitation into left ventricle per minute.

The right anterior cusp had a large hole 5 mm diameter with a small fold which may have acted as a secondary valve to the hole. The left anterior cusp had a hole about 4 mm diameter. The edges of both holes were thickened. The posterior cusp had an almost healed hole of about 1 mm diameter only.

The heart was only weighed after regurgitation had been tested, which was by mistake carried out with water instead of saline, so that some water passed into

TABLE 2  
Experiment B—*doc 15*

February 25	46 6	53 9		Slight capillary pulsation in mouth
April 12 and April 12	{ 51 8 51 6	58 6 57 8	0 4870 0330 0800 0510 2320 1860 1920 0230 0330 2630 2670 276 0 4930 0360 0910 0440 2260 2040 1970 0050 3220 2550 2900 280	After records taken second operation with damage to at least 2 cusps and some to molar.
June 20 and June 23	{ 67 2 64 5	63 0 64 6	0 5040 0550 0 0960 0690 252 0 5270 0570 0980 0660 243	After operation still under ether (see fig 3)
September 29	{ 76 7 66 3	574 0 574 0	0 0570 1090 0760 2170 2189 1990 0120 2860 1860 2880 263 0 0550 1070 0680 2380 2170 2030 0080 3140 2230 2860 268	\ rays under ether (see fig 2) Pulse rate 123 Atropine sulphate, 12 mgm. Pulse rate 206
September 30	{ 61 4	77 1 77 7		
December 12	{ 69 8	78 5	0 6080 0610 1140 0900 2400 2190 2330 00750 3080 2050 2800 299	With shivering pulse rate rose to 194 per minute Morphine 150 mgm. given for x rays

\* Right vagus cut in the neck in an unsuccessful attempt to produce a permanently rapid heart rate

the coronary arteries and some degree of water turgor resulted. The heart then weighed 177 grams and after 9 days in formalin (10 per cent) 164 grams. Controls on several normal hearts treated in a similar way suggested that the real weight of the heart was at least 135 to 145 grams, corresponding therefore to a minimum of 11 to 12 per cent of the body weight. The left ventricular wall was very

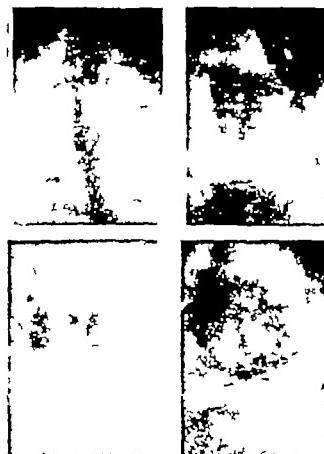


FIG 2 EXPERIMENT B

Two upper photographs are those taken on February 14, 1924, after morphine injection before any operation, the two lower are the anteroposterior and second lateral taken on September 30, 1924, after morphine and ether. Areas—Anteroposterior 48.0 and 66.3, lateral 48.4 and 77.7 sq cm.

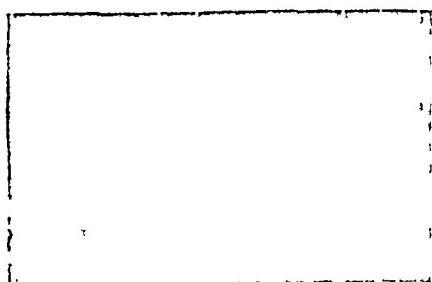


Fig 3a

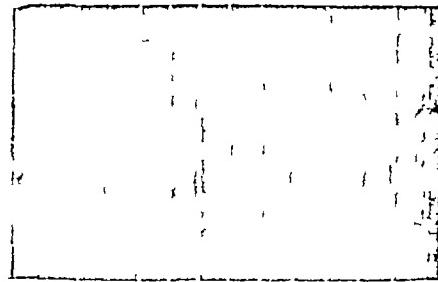


Fig 3b

FIG 3 EXPERIMENT B

*a* Electrocardiogram (white) Lead II and heart sounds (black) taken on April 12, 1924 when moderate aortic regurgitation was present

*b* The same records taken about 30 minutes later after operation with extreme regurgitation, and loud diastolic murmur

much thickened, the right was definitely thicker than normal, and the left auricle also thickened. There was no change noted macroscopically in the large arteries.

Casts showed that the left ventricle had a normally shaped conical cavity of 27 cc volume while the right had a much elongated spiral shaped cavity of

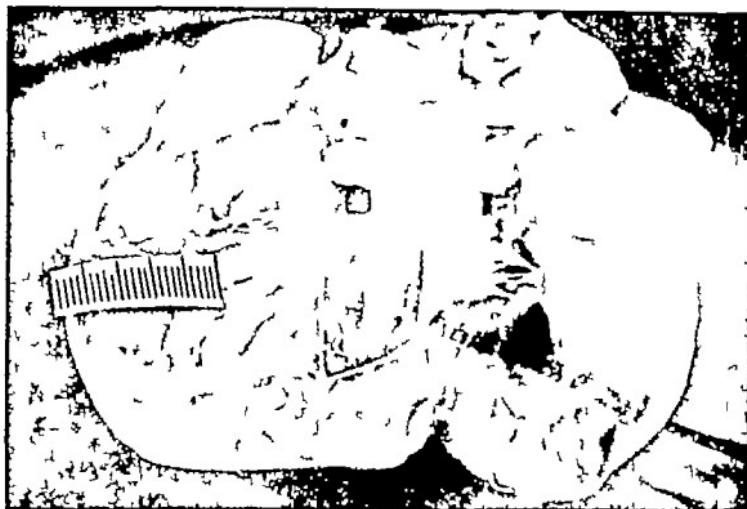


Fig. 4a



Fig. 4b

#### FIG. 4 EXPERIMENT B

*a* Heart at autopsy with glass rods inserted through main holes Millimeter scale on heart wall

*b* Casts of the ventricular cavities—the left conical, the right very elongated. *A* is the aortic valve *P* is the pulmonary valve *M* is the mitral valve

19.5 cc only. Figures 4 (a) and (b) demonstrate the valve lesions, and show the casts obtained. The ratio of ventricular weights by Lewis' method L/R 2.8—the normal value being 2.16 (Dr Grant).

*Experiment C—Dog 23*

Male, 10.5 kilos. Operated on while under the effect of atropine. Attempts were made to tear two valve cusps, at the end of the operation the pulse became quite weak and then gradually improved with an extreme water hammer.

Figure 5 illustrates the changes in heart size demonstrating that there is definitely no dilatation, in the clinical sense, under such conditions in spite of a big lesion and the feeble pulse existing shortly before the photographs were taken. There was unquestionably an increase in the heart shadow following the operation.

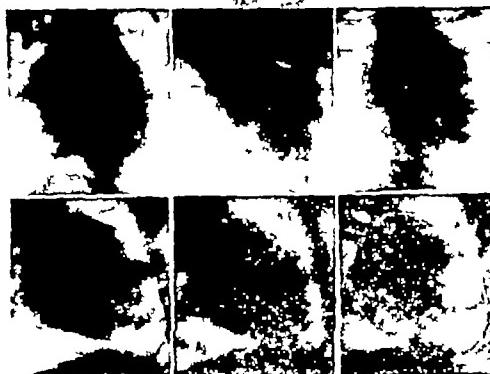


FIG 5 EXPERIMENT C

The antero-posterior photographs were taken (from left to right) with morphine 50 mgm., with atropine sulphate in addition 11 mgm., and immediately after operation with regurgitation still under the effects of the above drugs and recovering from ether anesthesia. The pulse rates were 48, 200 and 160 respectively. The lateral photographs were similarly obtained. The areas were antero-posterior 43.3, 34.8 and 36.8, lateral 46.1, 37.4, 42.1 sq. cm., and the lengths of the long axis 8.2, 7.5, 8.0 and 8.9, 8.4 and 8.7 cm.

particularly in the lateral photographs, but it was not outside the range that might be caused by the observed slowing of the pulse.

The electrocardiograms showed an immediate change, the Q wave becoming exaggerated in all three leads and the S wave which was originally present in Leads II and III disappeared, while the potential of R was exaggerated. Figure 6 shows the changes in Lead I and III, Lead II both before and after operation being very similar to Lead III. The slowing of the pulse was probably not due to the end of the atropine effect since the whole operation was completed quickly.

On the first day after the operation the animal was kept in a small cage

seemed fit and took its food well. On the following day it still seemed fit, was wagging its tail and was then allowed to run about in a large room. The pulse was, however, very collapsing and irregular, the rate varying from 140 to 160, and the respirations were fast and rather gasping. Shortly after this, its respirations became progressively faster and more laboured, it coughed up frothy fluid tinged with blood, and at midday died with lung edema.

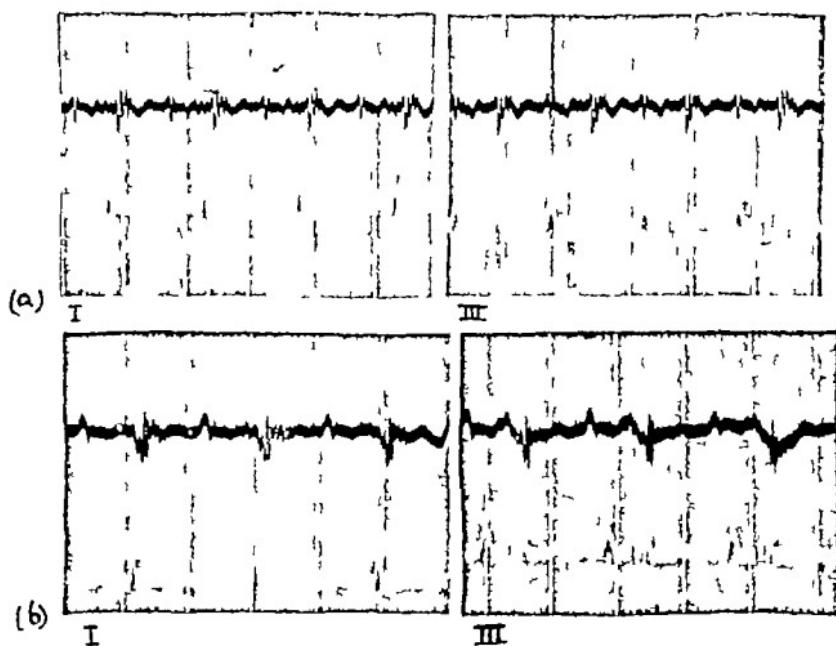


FIG 6 EXPERIMENT C

*a* Electrocardiogram (white) and heart sound record (black) taken immediately before operation morphine 50 mgm, atropine sulphate 11 mgm, Leads I and III

*b* Electrocardiogram (white) and heart sound record (black) taken about 30 minutes later immediately after operation when recovering from ether

*Autopsy.* Lungs Edematous and congested Liver Congested and firm Heart Weight 77.5 grams = 0.74 per cent of body weight The right anterior cusp had been torn until a mere shred was left, the left anterior cusp had a hole 2 mm in diameter and the posterior cusp one 3.5 mm in diameter The left ventricle was in a condition of extreme rigor mortis the whole heart small (autopsy one hour after death) and there was no evidence at this time of gross dilatation The amount of leak was not measured but the aorta was perfused with water to

cause an even water turgor and casts were then made, giving volumes for the right ventricle of 7 cc and 6.25 for the left L/R ventricular weight ratio 2.1 (Dr Grant)

*Experiment D—Dog 18*

Male Originally 9.25, finally 13.0 kilos in weight A number of electrographic records and x-rays taken before and after operation all under morphine

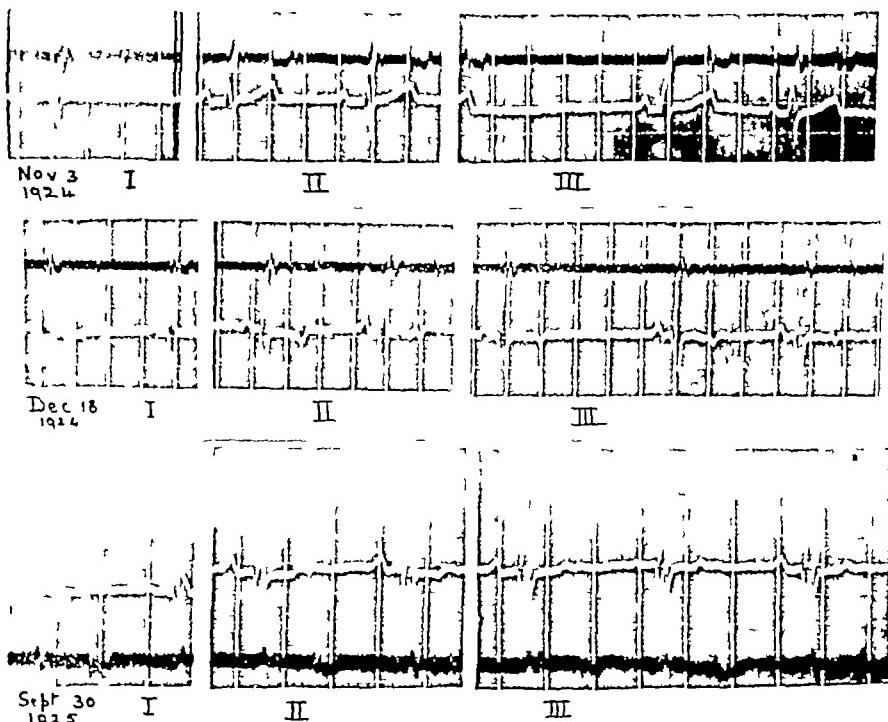


FIG 7 EXPERIMENT D

Electrocardiograms (white) and heart sound records (black) of dog 18, all after morphine, above before any operation, in the middle 6 weeks after the initial operation and below 11 months after the initial and 9½ months after the second operation

(see fig 7) The animal was excitable and on the last two occasions had to have ether in addition Records were also obtained after atropine injections

November 7, 1924 Operated on with perforation to two valves, and immediately after operation extra systoles were noted usually of left ventricular type but with occasional contractions apparently originating in the right ventricle

December 18 1924 Reoperated with attempted stretching of old holes Sound caught in a valve, met considerable resistance and was eventually forced through, but doubt was felt as to actual position of the stilet which was withdrawn rapidly as the heart action became very feeble The dog was exercised at intervals between April 3 and September 30, 1925, and during this time the amount of work it was able to do increased For example the average work done on 5 occasions between April 27 to May 5 was 2 58 Kilometers with a climb of 450 meters, while the average of 12 occasions between August 26 to September 20 was 3 kilometers with a climb of 524 meters During warm weather in July the average work on 7 occasions was only 2 01 kilometers with a climb of 350 meters and twice work had to be stopped, as the animal vomited while working

The correlation of the change in the shadow of the heart with lengthening of the Q-S interval is shown in the following tabulation

Date	X-ray area with morphine		X-ray area with morphine and atropine		Duration of Q S interval
	A.P.	Lat	A.P.	Lat	
November 3 1924					
November 5 1924	38 4(100)	43 8(100)	34 0(100)	38 6(100)	0 056(100)
November 7 1924					
First operation					
December 18 1924	48 0(125)	48 3(110)	42 2(124)	41 6(108)	0 067(120)
Second operation					
March 30 1925	49 8(130)	51 4(117)	44 6(131)	46 4(120)	0 074(132)
September 30 1925	52 2(136)	51 4(117)	47 5(140)	47 1(122)	0 079(141)

\* Bracketed figures indicate percentage change

*Autopsy* October 8, 1925 Blood pressure 170/76 in brachial and 252/70 in femoral (after ligation of other femoral) with pulse of 109 Animal killed with ether Heart weight 105 grams (0 81 per cent body weight) The leak was 1 75 liters per minute Casts gave volumes for the right ventricle of 29.3 and for the left of 34.7 cc

The left ventricular wall was from 10 to 20 mm thick and the right 6 mm Dr Grant reported 'in the posterior cusp was an oval hole about 5 mm in diameter with thickened edges. The left anterior cusp was thickened at its base and on its aortic surface a small perforation the size of a pin's head was seen in the center of the thickened base This hole led into a false aneurysm of the ventricular wall, underlying the thickening described It was lined by a thick layer of connective tissue. The mitral valve was thickened and showed irregular flat thrombi possibly implying a low grade inflammation though no organisms

TABLE 3  
*Experiment E—dog 4*

No morphine									
Morphine 45-120 mgm									
Date, 1923	area sq in. 2	Recpiration area sq in. 2	T.P.	S.T.	Cle	P.R.	S.	E.	K.
January 17 und January 23	{ 71.0 68.1 67.0 67.8 66.7 69.3 71.4	{ 1 vp 1 vp 1 vp (?) 1 vp (?) Insp	{ 7-1 2-1 3-0 2-0 1-6 0-? 5-0	{ 73 85 60 65 56 81	0.9770 0.0410 1.2300 0.0590 0.2210 0.2300 0.2380	0.0330 0.2240 0.1640 0.2330 0.1660 0.0920 0.0020	0.2380 0.2240 0.1640 0.2330 0.1660 0.0920 0.0020	0.2380 0.2240 0.1640 0.2330 0.1660 0.0920 0.0020	0.2380 0.2240 0.1640 0.2330 0.1660 0.0920 0.0020
January 23 Operation—2 cusps injured									
January 25	{ 61.0 58.2 62.3 60.0	{ Exp ? Exp ? Exp ? Exp ?	{ 2-0 4-5-2 0-0 0-0	{ 0-1 82 107 141	0.5710 0.0540 1.0000 0.0490	0.0000 0.2340 0.1870 0.0230	0.0000 0.1870 0.1870 0.3100	0.0000 0.0280 0.0280 0.0450	0.0000 0.2560 0.1750 0.2470
January 31 February 9	{ 65.0 71.6	{ Exp ? Lvp	{ 4-0 2-5-0	{ 99 102	0.4400 0.5250	0.0320 0.0470	0.0930 0.1000	0.0530 0.0600	0.1700 0.2130
March 6	{ 70.5 73.2	{ Lvp (?)	{ 1-0 0-?	{ 102 119	0.7000	0.0490	0.1150	0.0600	0.2340
April 10	70.5	Insp	0-1	92	0.5420	0.0500	0.1090	0.0560	0.2230
April 16 Operation—3 valve cusps injured									
April 18	{ 71.0 71.2	{ Insp (?)	0-0	115	0.5260	0.0420	0.0830	0.0430	0.2400
April 20*	76.8	Insp	0-0	160	0.3710	0.0510	0.0990	0.0520	0.1930

were found." The autopsy therefore makes it doubtful whether more than one valve was perforated at either operation.

*Experiment E—Dog 4*

Male, 14.75 Kilos Electrocardiographic records were taken with and without morphine and 47 short exposure x rays were taken after morphine with a few exceptions. A few typical figures of the x ray shadow areas with morphine and of the time relations of the heart cycle without morphine are given in table 3.

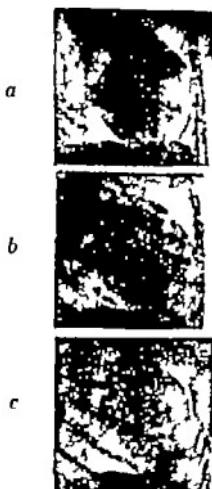


FIG 8 EXPERIMENT E

Short exposure x rays (a) x ray without morphine, 3 days before second operation Area 68.8 sq cm Long axis 11.0 cm Expiration S 0—T 0.5 Pulse rate 85

(b) With morphine 2 days after operation Area 70.0 sq cm Long axis 11.4 cm Respiratory phase uncertain S 0—T 1 Pulse 114

(c) Without morphine 4 days after second operation just before death Area 74.2 sq cm Long axis 12.1 cm Inspiration S 0—T 0 Pulse 158

Following the first operation there was a hematoma in the neck, from which a clear fluid drained. The second operation was unusually prolonged owing to a breakdown of the fluoroscope and the trauma great. In spite of extensive damage to three valve cusps the dog seemed quite fit on the following day, though quieter than usual. By the fourth day after operation the animal was obviously extremely ill and feverish, and died in the afternoon, about 1 hour after the last record was taken.

During the first operation the fluoroscope showed a lengthening of the shadow

from 97 to 104 mm with a diminution of the transverse diameter from 92 to about 78 mm The breakdown of the x-ray machine prevented such observations at the second operation, but lateral and antero-posterior photographs taken two days later (see fig 8) demonstrated that such a lengthening had occurred

Autopsy showed a left sided pneumonia, haemorrhagic myocarditis and a slight pericardial effusion Weight of heart 128 grams (0.87 per cent of body weight) Regurgitation occurred at rate of 3.6 liters per minute

There were four lesions in the three cusps, one cusp having two holes, and each of the others one Two holes had fibrosed edges and a diameter of about 3 mm, two appeared recent and had diameters of 5 and 3 mm Culture of heart's blood showed the presence of haemolytic staphylococcus aureus

The left ventricular wall seemed slightly thickened L/R ratio of 3.8 (Dr Grant)

#### *Experiment F—Dog 13*

Male Initial weight 12.5, final 17.00 kilos<sup>2</sup> No growth, but initially thin It was allowed to take what exercise it liked on the machine on 28 occasions between December 17 and February 4, remaining on the machine for 30 minutes to 1 hour It covered a distance averaging 3.36 kilometers with a climb of about 700 meters Even before operation this work had some effect on the heart size During the first three weeks of the experiment the dog had been kept in a small cage without any exercise and the areas of the heart shadows (long exposures) had decreased 4.3 per cent in the lateral and 7 per cent in the anteroposterior photographs By January 25, after this period of exercise the shadows had increased to the original size and by February 4th they were further increased above this size (lateral 4.4 and anteroposterior 10.0 per cent)

On February 4, the animal was operated on and extensive damage caused to two cusps On the following day extra systoles of right ventricular type were observed Seventeen days after operation exercise was again commenced The dog panted much more than previously, took more frequent rests, would not gallop of its own accord, though it could do so when encouraged by food At the end of such periods it could not be made to work without great persuasion, was very short of breath, and the apex beat and pulse, which originally were bounding, became almost imperceptible In spite of this on removal from the machine, it would run off to a good meal as usual During this period it was exercised 4 or 5 times a week and covered on the average 2.05 kilometers with a climb of about 470 meters in 35 minutes, after which it became fatigued and was removed Three and a half weeks after operation it resumed galloping of its own accord, but would only continue this for a short while at the commencement of its work

Five weeks after operation the heart shadow was still slightly below the original value, and 8 to 11 per cent below the figures obtained just before operation

At this time it was operated on again and died 5 days later of acute septic

endocarditis Two days after operation it resembled dog 4 (experiment E) in that it was relatively unaffected by the large lesion Later it also showed an ante mortem dilatation

Six to seven hours before death electrographic records showed no extra systoles, two hours before death they were again present but of a different type to those seen after the first operation (Lead II only)

*Autopsy* Weight of heart 155 grams (0.91 per cent body weight) Leak 3.5 liters per minute. One cusp had a large hole with thickened edges, another had two holes both very large and one thickened and old. Vegetations were present on both old holes and on the mitral valve, which had also been damaged On the posterior surface of the left ventricle there was a suppurating infection almost resulting in perforation of the wall

#### *Experiment G—Dog 14*

Male. Initial weight 16.5, final 21.5 kilos Increase due to fat Composite x ray photographs with morphine and E C G records without morphine. Before operation x ray shadows averaged antero-posterior 64.7 and lateral 73.3 sq cm E C G cycle 0.457, QS 0.042 and QT 0.200 second.

July 25, 1924 Operation with puncture of two cusps.

March 3 to 30 Exercise on 20 occasions with average distance of 3.2 Kilometers and climb of 708 meters in 45 minutes. On one occasion 5.5 Kilometers with climb of 1220 meters was covered in one hour

April 11 X ray—antero-posterior 82.5 and lateral 89.3 sq cm E C G cycle 0.475, QS 0.051 QT 0.207 second A second operation attempted and the aorta was perforated causing death

*Autopsy* Holes 3 to 4 mm diameter with thickened rims in both posterior cusps. The left ventricular cavity appeared large, but walls not unusually thick. Weight of heart 175 grams (0.81 per cent of body weight) L/R ratio 2.7 (Dr Grant)

#### *Experiment H—Dog 16*

Male. Initial weight 14.5, final 13.5 kilos and became very thin through vomiting Records as in experiment G Animal very athletic, and able to jump out of all usual cages. Initially x ray shadows—antero-posterior 60.6, lateral 67.7 sq cm E C G cycle 0.938, QS 0.050 QT 0.268

March 6 1924 Section of right vagus in chest just below recurrent laryngeal (We are indebted to Dr Sweet for the performance of this operation)

March 22 X ray—antero-posterior 56.6 lateral 64.3 sq cm E C G cycle 0.560 QS 0.044, QT 0.234 Section of left vagus.

April 2 Vomiting frequently during last week. X ray—antero-posterior 59.6, lateral 58.6, pulse 88 at this time (under morphine) E C G cycle 0.499 QS 0.054, QT 0.243 Operation—damage to 2 cusps. X ray—antero-posterior 53.0, lateral 60.3 sq cm. Pulse rate at this time (with morphine) 112

April 3 X-ray—antero-posterior 53 1, lateral 54 6 sq cm E C G cycle 0 488, QS 0 051, QT 0 228

April 5 E C G cycle 0 501, QS 0 045, QT 0 224

April 6 Died apparently from weakness secondary to vomiting

*Autopsy* Right vagal stumps included in scar tissue, and one branch to heart coming off above section Lungs edematous and congested Liver "nutmeg" in appearance Regurgitation at 2 2 liters per minute with 1 meter pressure of saline Large holes in two cusps Heart weight 132 grams (0 91 per cent of initial body weight) L/R ratio (Dr Grant) 2.3

#### *Experiment I—Dog 19*

Male Initial weight 14, final 18 6 kilos (Initially very underweight) X-rays and E C G records all after morphine

January 22 Before operation—x-ray shadows—antero-posterior area 62 6 sq cm Length 9 6 cm Lateral 68 9 and length 10 9 (Pulse rate 60) After atropine sulphate 20 mgm Antero-posterior 57 1 Length 9 9 Lateral 57 2 sq cm Length 10 5 cm (Pulse rate 225) E C G (one occasion only after morphine) cycle 0 978, QS 0 071, QT 0 302 Operation—damage to two cusps

January 24 X-ray shadows—antero-posterior 58 4 sq cm length 9 8 cm Lateral 64 3 length 10 8 (Pulse rate 115) After atropine sulphate—antero-posterior 52 5, length 9 2 Lateral 53 1, length 9 9 (Pulse rate 220) E C G Cycle 0 714, QS 0 055, QT 0 278

February 20 Developed partial paralysis of both hind limbs following a fight

February 25 Paralysis not improved X-ray shadows—antero-posterior 69 0 sq cm, length 9 9 cm Lateral 73 3, length 11 3 (Pulse rate 126) After atropine sulphate antero-posterior 67 0, length 10 6 Lateral 65 0 sq cm length 11 0 cm (Pulse 205) E C G cycle 0 767, QS 0 056, QT 0 286 Killed with chloroform

*Autopsy* Heart Holes about 3 mm in diameter present in both posterior cusps Left ventricular cavity 41 3 cc right 26 2 cc Weight of heart 144 grams (0 77 per cent of final body weight)

Spinal cord Evidence of embolism in lowest sacral segments Macroscopic examination did not demonstrate a higher lesion such as was indicated by the symptoms before death

#### RESULTS

##### *Signs and symptoms*

An immediate increase in pulse rate was the invariable accompaniment of the production of a lesion in our animals, except when the pulse rate was already high as the result of an atropine injection, when on the contrary there was a slight, but quite definite slowing The

following day and during the next few months the pulse rate gradually slowed, but never returned to its previous low level, whether observations were made with or without morphine. The slowing of the pulse by morphine was much less noticeable after the operation. In every case the pulse was of a water hammer type of varying degree but often extreme.

There was no obvious disability, nor even, as will be seen later, any great loss of power to do work as the result of a single operation with damage to one or two valve cusps. With damage to three valves, if performed in two stages, a stranger would have considered the dog absolutely normal even on the first day after operation, though we could recognize that the dog's expression of pleasure at our entering the room was less violent. In strong contrast was the immediate death of dogs, which occurred if lesions of this extent were made at a single operation.

The protocol of experiment B gives an example of an enormous change in pulse rate at such a second operation without any other obvious symptoms, and the photograph reproduced indicates the magnitude of the lesion. Immediately after the second operation before the partial healing of the one cusp, the lesion must have been even more severe.

The general signs of aortic regurgitation such as diastolic murmurs (always audible and sometimes recordable photographically), precordial thrill, capillary pulsation, and the so-called differential blood pressure were commonly present.

The differential pressure was measured in two animals which had had an aortic lesion for nearly 1 year, the difference amounted to only 16 mm in one case (experiment B) and to 82 mm in the second (experiment D), but in the latter the other femoral had been tied, so that the difference was somewhat exaggerated (21). There was, therefore, no evidence of any marked contrast between the acute and chronic animals (21). No macroscopic evidence of arteriosclerosis could be detected. No capillary pulsation could be detected in the mouth during the first 24 hours, but it was usually present on the second day, the slow development agreed with Lewis's (23) conception of a slow development of vasodilatation. In our experiments, however, no such definite deduction would be warranted after liga-

ture of one or both carotids Decompen-sation of gradual onset was only seen in one animal (experiment C)

#### *Electrocardiographic changes*

There was no regularity in regard to immediate changes in the electrocardiograms In some animals there was at once an exaggeration of the Q wave and diminution of the S in all leads (experiment C), but often no such immediate change in Q or S was recognizable Usually the P wave was exaggerated and the extreme potential differences reached in the QRS group increased, but this was not constant and there was considerable variation in the alteration of any one wave

On the other hand in the records obtained after the survival of the animal for some time the changes were very consistent The potential of the P wave was nearly always increased, particularly in Lead II and III and its duration was greater The Q wave had a much increased potential in all three leads The potential of the R wave was less constantly changed, but again was commonly increased in all leads The S wave became diminished in Leads II and III, no S wave was noticed in Lead I, either before or after operation The changes in the T wave were irregular, the potential might be increased, decreased, or the wave might be inverted; very commonly it was a double wave, both before and after operation The changes in the character of waves in such chronic animals are illustrated in figure 7 representing the changes seen in experiment D The ultimate changes seen in the electrocardiograms, except in the duration of the waves, were very similar to those which sometimes occurred immediately after operation Both would be explicable as the result of a rotation of the heart (28) It should be pointed out that the bizarre curves seen in figure 7 (notching of the R and duplication or inversion of the T wave) probably have no special significance, such variations are not at all uncommon in apparently normal dogs There has never been any evidence of one-sided preponderance of the type described clinically The parallelism between the changes in heart size and the average duration of the Q-S group typical of that occurring in the whole series is noticeable in the records of experiments D and G A similar parallelism is seen in table 2 and to a

less extent in table 1 (contrast experiment I), it is the more surprising since any individual measurement of the Q-S group may have a serious error, since the electrical changes in the two ventricles may neutralize and mask one another.

Extra systoles of ventricular origin were seen immediately after operation or within the next few days in 8 out of the 11 survivals, and in every animal that was examined at all frequently after operation. In nearly every case they were of left ventricular origin and occurred in groups of 4, 5 or more, in one animal (experiment D) premature left ventricular contractions occasionally alternated with others of right ventricular type immediately after the first operation, but the autopsy findings are difficult to interpret. In experiment F extra systoles of right ventricular type occurred on the day following the initial operation. In the same dog several days after the second operation and just before death from infection extra systoles were again seen and though only recorded in Lead II, they were of quite a different type from those seen earlier.

#### *Time relations of the cardiac cycle*

In general the changes observed in the duration of systole and diastole were the same in both electrical and mechanical records, whatever differences between the two occurred were mainly seen in the first few days following operation. Following aortic regurgitation systole was relatively long for the pulse rate existing at the time. This may be readily seen in the figures given for experiments A, B and E by the changes in the factors K and M already described (19), which represent the relation of systole to the square root of the cycle. In the earlier weeks after operation the lengthening is partly in the S-T interval and causes an increase in the value K' as well as in K, later with the development of hypertrophy the Q-S interval is increased (see experiment D), and the S-T interval sometimes returns to a normal length, K being still increased, but K' increased or normal (experiments A, B and E). The changes of the mechanical systole follow the same course as those of the electrical, except immediately after operation when the relationship of the T wave to the second sound is very variable, and consequently the two figures do not necessarily go always in the same direction. The discrepancy

between the two which may occur even under normal conditions after morphia is well demonstrated in figure 7. The values given in table 3 without morphine suggest that some factor may make the T wave outlast the second sound particularly just after the production of an aortic lesion. Of six animals where a series of records were taken without morphine four showed a T outlasting the second sound to an unusual extent during the first few days after operation. It did not appear to be dependent on excitement.

There was also seen at times an apparent discrepancy between the first sound and the QRS group, after operation a large abrupt vibration was sometimes seen which would have been considered the beginning of the ventricular first sound, if it had not occurred considerably before the Q wave. Such sounds were presumably auricular in origin and were disregarded in estimating mechanical systole.

Diastolic murmurs though audible were only occasionally recorded clearly, sometimes they were most evident in early diastole, sometimes in late diastole, almost merging with the first sound. The records reproduced in figure 3 from experiment B show in (a) a small early diastolic murmur due to the lesion produced at the first operation 7 weeks earlier, in (b) obtained immediately after the second operation there is a very definite murmur extending throughout diastole up to the first sound. The pulse rate had risen as the result of the operation from 122 to about 200 and in record (b) the T and P waves are partly fused.

#### *Changes in heart size*

In another paper (19) the variations in heart size seen in these dogs under normal conditions have been discussed, and it will be seen that changes in size beyond the experimental error are demonstrable by x-ray after operation.

*Immediate changes* The fluoroscope demonstrated at the time of operation an immediate change in the shape of the heart. Almost invariably measurements on the screen showed a lengthening of the heart shadow usually accompanied by a diminution of the transverse diameter. On the other hand in one case, which involved damage to three cusps within a few minutes, a marked increase in both diameters was observed, the length increasing from 89 to 102 mm and the

breadth from 74 to 79 mm. The animal, however, died about five minutes later with a terminal ventricular fibrillation. At autopsy one cusp was partially detached, and may have possibly obstructed the right coronary orifice. In one other animal, with only an average amount of damage to two cusps, dilatation was demonstrable immediately after operation, the heart area being increased above the normal value by 2 per cent in the antero posterior photograph and by 17 per cent in the lateral. The animal was dying and at the time of examination no pulse could be felt, though it was still breathing. This was the minimal valve lesion which caused immediate death. In surviving animals there was no visible dilatation and occasionally none might be demonstrable even shortly before death. For instance in one other animal which died on the table from a lesion to three valves at a single operation, the shadow only lengthened on the fluoroscope from 84 to 91 cm., while its transverse diameter was reduced from 81 to 60. This absence of dilatation in the clinical sense has been most striking. Experiment E illustrates the degree of change usually observed with the fluoroscope in animals which survived.

*Early changes.* Immediately after operation, the following day, and generally at any time during the first week, the heart shadow in diastole was smaller than any systolic area before operation. This is clearly seen in the records of experiments A and E. Occasionally the photographs taken within a half hour of the operation showed the lengthening already described, more often it was slight and certainly less marked than that seen by fluoroscope during the operation. In experiment E there was seen a decrease in the diastolic heart shadow two days after the first operation (table 3), which amounted to about 13 per cent and even after a week was still 7 per cent. If the diminution were due to the pulse rate change alone, and the curve followed was similar to those of Meek's animals (24), only a diminution of 5 to 8 per cent on the first occasion and of 2 per cent on the second might have been expected. With this diminution in size there occurred an initial lengthening of the heart demonstrable on the fluoroscope, though x-ray photographs taken on the following day did not show it. After the second operation, when the leak was enormous, the antero posterior shadow was slightly increased even

though the pulse rate was faster, the lateral photographs (see fig. 8) showed a more definite increase, and the lengthening was now obvious

*Late changes* After a short while, a progressive but slowly developing heart hypertrophy became evident. This may be observed in the values already given for experiments A, B, D and E and the hypertrophy is confirmed by the weight of the heart at death, as well as in experiments B and E by the ratio of the weights of the two ventricles. On the other hand, it is noticeable that such a hypertrophy may be masked in x-ray shadows by the effect of the pulse rate change. In experiment E there was in our opinion a hypertrophy, though the actual areas of the heart shadow merely showed a gradual recovery of the original size (if the last day before death be excluded). The later areas, however, were obtained with a pulse rate of about 115 and were approximately as large as those obtained with a pulse rate of about 70 before operation, with the change in the pulse rate a smaller heart shadow might be expected and its absence demonstrated the hypertrophy.

The effect of such pulse rate changes can be avoided by the comparison of shadows obtained when the animal is under the effect of atropine. Data so obtained have been given for experiment D. Experiment B showed the greatest change in the heart shadow that we have observed, and yet even after atropine injection the shadow area remained much above the value originally found with a slow pulse, unfortunately atropine was not used in this case previous to operation.

*Ante-mortem dilatation* A dilatation, demonstrable by x-rays, is present in dogs with an acute infection just before death (experiments E and F) but the great lengthening of the heart seen in experiment E was not a constant finding.

#### *Ability to do work*

Some of the animals were tested on the treadmill already described. In order to be able to make a rough comparison of the capacity of the aortic animals with that of a normal animal, a single dog (experiment F) was allowed to do work both before and after operation. This experiment suggests that the amount of work that an animal

can do may be reduced, even though a superficial examination does not demonstrate any disability. On the other hand, the amount of work done after operation was surprisingly great, considering the extent of the lesion and the probability of the existence in this animal of a subacute infection dating possibly from the original operation.

The absence of any obvious disability was also well demonstrated by dog 14 (experiment G), which did more work after operation than did dog 13 before operation.

The protocol of experiment B, that has been given already demonstrates that with a considerable leak as the result of two operations the ability to do work may be much more reduced, and under these conditions a high room temperature had a much more depressing effect, and signs of discomfort and vomiting were then often seen. The data of experiment D demonstrate a similar effect, though to a less degree, and in this case the symptoms disappeared during the last month of life. The vomiting, occurring during the work or soon after in experiments B and D, appeared to be associated always with over fatigue and dyspnea.

#### *Gross pathology*

Examples have already been given which illustrate the type of change observed at autopsy. There seems to be no doubt that after a few weeks or months the weight of the heart is somewhat increased so that heart weight to body weight ratios greater than those considered normal by other workers have generally been observed. This ratio is so variable in dogs, that the figures obtained in any particular dog are rarely outside normal limits, but the consistent high ratios are significant.

The figures given by Joseph (29) show quite definitely the variation of the ratio in supposedly normal dogs with varying weight and demonstrate that the normal ratio for male dogs of the smallest size that we have used (9-10 kilo) would be about 0.72 per cent, while for the larger animals (e.g., dog 14) it would be less and probably about 0.64 per cent, comparison should, therefore, be made after considering the absolute weight of the dog.

Table 4 will demonstrate the difficulty of judging by mere weight ratios.

The average weight of the first group was 12.31 kilo and the average ratio 0.796, the corresponding values for the second group was 15.15 kilo and 0.86. If, however, comparison is made with only the first 5 dogs of the second group, in order to have comparable body weights (average weight of the 5 dogs 12.64 kilos), the ratio observed after

TABLE 4

Number	Weight	Ratio	Leak per minute	Survival	L/R ratio	Casts		Remarks
						L	R	
Acute death or short survival								
20	9.25	0.81	liters			9.7	27.5	Probably rigor at autopsy
23	10.5	0.74		3 days	2.1	6.25	7.0	Rigor at autopsy
21	13.0	0.82				23.7	22.8	(See fig 9)
22	14.3	0.70		4 days		21.0	26.7	
16	14.5	0.91	2.2	4 days	2.3			Athletic
Average	12.31	0.796		2 days	2.2			
Longer survival								
3	10.0	0.91	2.3	6 weeks	2.8	27.0	19.5	Exercise (see fig 4)
15	12.25	1.15(?)	2.8	10 months				
18	13.0	0.81	1.75	11 months		34.7	29.3	Exercise
17	13.25	0.88		6 weeks	2.8	29.0	20.0	Large holes in two cusps
4	14.7	0.87	3.6	3 months	3.8			
7	17.0	0.65	2.7	4½ months	2.9			
13	17.0	0.91	3.5	5 weeks	3.0			
19	18.6	0.77		1 month	2.9	41.3	26.2	Exercise Large holes in two cusps
14	21.5	0.81		11 weeks	2.7			Exercise Large holes in two cusps
Average	15.15	0.86		4 months	3.0			

the existence of a lesion for an average of  $5\frac{1}{2}$  months is 0.92, demonstrating the considerable change which results from operation. The danger of drawing too definite conclusions from individual ratios is well illustrated by dog 7, which showed a heart weight ratio little, if at all, above the normal average yet the heart shadow (anteropos-

terior) increased 6 to 7 per cent above the area observed before operation in spite of the pulse rate rising from 61 to 103.

The ratio of the weight of the left ventricle as compared with the right was determined according to Lewis's method by Dr Grant in some of the dogs (see table 4). The ratio found by him in 5 normal dogs averaged 2.16 with extremes of 1.9 and 2. Of the two animals with aortic lesions showing a ratio within the normal limits dog 16 (experiment H) survived the aortic lesion for only 4 days, dying from continued vomiting secondary to vagal section. This dog was obviously athletic, and in agreement with this history

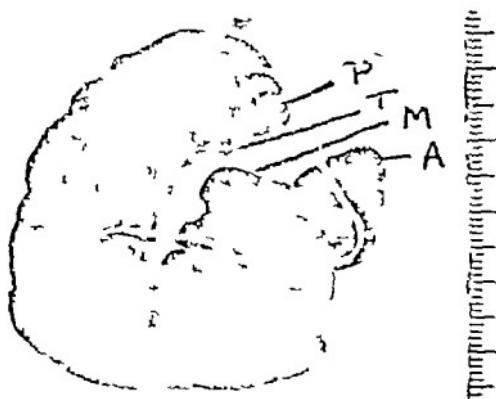


FIG. 9. Casts of ventricle in dog 21 (table 4) which died on operating table giving normal relationship of ventricles. Letters as in fig. 4(b), T is tricuspid valve.

the heart at autopsy was large but the hypertrophy was symmetrical. All the other animals showed a definite increase in the relative weight of the left ventricle even dog 7 which showed the low heart to body weight ratio. Some correlation between the degree of leak and the abnormality of the left to right ventricular ratio can be seen in the table.

This increase in the weight of the left ventricle may perhaps be correlated with an increase in its cavity as compared with that of the right. This increase was obvious at autopsy, and was measured by casts in a few cases (see table 4 and compare figure 4b from dog 15 with figure 9 from dog 21). Agreement between the values for the

cavities of the two ventricles was only seen in operated animals if they died immediately or shortly after operation. The casts obtained a few days after the production of the lesion show that if there is an immediate dilatation of the left ventricle following the lesion, it is not demonstrable at autopsy.

The occurrence of infection has been already mentioned (experiments E and F) with the appearance in experiment F of a localized necrotic area in the left ventricular wall, which may have originated as an infarct. It was noticeable that a few hours before death numerous extra systoles were observed. Extra systoles of left ventricular type were common soon after operation, when damage to the left ventricular septal wall probably occurred and evidence of which could sometimes be found at autopsy, occasionally extra systoles of the right ventricular type were seen.

The only other complication observed was the appearance of partial spinal paralysis of both legs in experiment I.

#### GENERAL DISCUSSION

One of the most definite changes was an immediate increase in pulse rate, the heart action slowing again somewhat as the heart hypertrophied, but never again regaining its previous low level. This change in rate has either not been observed, or where observed, in our opinion, has not been given sufficient attention in the work of previous writers. In acute experiments the figures given by H. A. Stewart (6) and the tracing reproduced by Wiggers (10) show little change in pulse rate and sometimes an actual slowing, this perhaps corresponds with the slowing of the pulse in our experiments, when the initial pulse was fast from atropine injections. It is conceivable that in any damaged heart, an impairment of the coronary circulation might occur sufficient to interfere with the heart's action. Hasenfeldt and Romberg noted a moderate increase in pulse rate following the operation but did not lay any stress on it. The tracings reproduced by Marey from experiments on the horse show an enormous increase in pulse rate entirely comparable to ours. The immediate occurrence of the change suggested some reflex effect on the heart. The factors causing variation in pulse rate have recently been investigated by Anrep and Segall (30). It seems unlikely that changes

in the cerebral pressure occur sufficient to produce the result observed, but if the reflex is of the Bainbridge type it can hardly be due to distension of the right side of the heart.

The initial diminution in heart size must be partly dependent on the change in heart rate. Figures have already been given, however, which suggest that the change is greater than that to be anticipated from this cause (experiment E). The values obtained in experiment H after vaginal section give a similar impression. The figures afford some indication of an actual increase in "tone" of the heart muscle and suggest that some change has taken place enabling the heart to do its work efficiently with a smaller initial volume. Therefore the contention of H A Stewart that the tone may be increased is sup-



FIG 10 Shadows of left ventricle obtained in normal dog immediately after death.

ported but certainly is not proved, since no determinations were made of the pressures distending the heart in diastole.

The x-ray shadows measured are produced by all the chambers of the heart and the left ventricle only contributes a small proportion. By injecting after death a strong solution of sodium iodide into a normal dog, x-ray photographs were obtained of the left ventricle after death which are reproduced in figure 10, the left ventricle was responsible for only 44 per cent of the shadow in the antero-posterior plane and for 60 per cent in the lateral. Diminution of the total shadow does not, therefore, necessarily exclude dilatation of the left ventricle.

If one considers a condition in which the pulse rate is changed by

about 100 per cent as the immediate result of the operation (as in experiment E) and if one supposes little change in the total circulation rate, then the right ventricle must expel only 50 per cent of the previous volume per beat, and a similar diminution must occur in the filling of the auricles. But the left ventricle is to some extent filled also from the aorta and may conceivably be distended to its normal value or even beyond this, in spite of the faster pulse, if the pendulum blood is of any appreciable amount. Against this possibility are the repeated observations of a diminution in heart size exceeding Meek's values, in favour of it is the common occurrence of an actual lengthening of the heart shadow observable on the fluoroscope, and sometimes, but not always, in photographs taken on the following day. Such a lengthening could be readily accounted for by an uneven filling of the two ventricles, so that the left ventricle was completely filled, while the right formed a lengthened narrow cavity lying along the cone-shaped left ventricle (compare experiment B). Such an hypothesis would also explain a tendency to a smaller decrease in the size of the lateral shadow than in the antero-posterior following operation, since the left ventricle would under such conditions be responsible for a larger share of the lateral shadow.

The question as to whether a real leak of a considerable quantity of blood back into the ventricle is possible must be considered. Stewart has rejected this hypothesis on heart plethysmograph values, but these have the same fallacies as have our x-rays. Wiggers has assumed the possibility of the occurrence of a regurgitation of pressure with only minimal exchange of fluid, a hypothesis which is difficult to comprehend, if the heart muscle retains its normal elasticity. A regurgitation of fluid lasting throughout diastole is indicated by the murmurs, which characteristically last the greater part of diastole and which in experiment B were recordable throughout this period. Actual measurements of the leak in our animals at autopsy demonstrate that with a pressure difference of 73 mm of Hg regurgitation would have occurred at the rates which might reach as high as 3.6 liters per minute. An approximate estimation of the actual leak during any diastole can, therefore, be made. For instance, in experiment B there was on December 12, 1924, a pulse rate of 99 and a diastole, measured from heart sound records, of 0.390 seconds.

At autopsy the leak was found to be 45 cc per second with a pressure of 1 meter of saline (73 mm of Hg). If the isometric relaxation phase (II) lasted 0.022 second there would remain 0.48 second during which regurgitation might conceivably occur. At the time of death the diastolic pressure was found to be about 50 mm. If the mean pressure difference between the aorta and left ventricle during the above period was 73 mm, the leak should have been 17.2 cc. if, however, this pressure difference was less and possibly about 30 mm (supposing mean pressure during diastole about 55 and the intraventricular pressure as 25 mm), the leak ought to be about 7.0 cc. Such a value for the regurgitation would be similar to the difference in volume of the two ventricles observed at autopsy. Taking Marshall's figures (51) for the normal output of a heart per minute in dogs and dividing by the pulse rate in this dog at the time one can anticipate that the normal output of the left ventricle per beat would be about 15.8 cc. Then any such leak would mean a penultimate blood nearly 50 per cent of the normal output of the ventricle and certainly not negligible.

If the values for dog 18 experiment D1 with a pulse rate of 97 be treated in a similar way a leak of 11.5 cc would be probable with a difference in pressure of 7 mm of Hg. The actual diastolic pressure just previous to death was about 70. The difference in the capacities of the two ventricles was 5 cc as measured by casts at autopsy, and a leak of this amount would be expected if the mean difference between the aortic and ventricular pressures during diastole was about 55 mm Hg. It is somewhat remarkable and perhaps a mere coincidence that the leaks calculated from the duration of diastole for a pressure difference of 30 or 55 mm. Hg are in both animals almost the same as the differences in the ventricular cavities.

A complete set of data is not available for any other animal since the rate of leak was often not measured in case that procedure might distort the casts. In experiment I (dog 19) the lesion was of such a size that a leak of slightly above the average say 3 liter per minute might be assumed. The differences between the ventricular casts exceeded that of any other animal amounting to 15.1 cc. The heart shadow was also much lengthened. This seems to be correlated with an unusually slow pulse (81) for an aortic regurgitation and with a

long diastole of 0.5 second, which would give a leak of 23.9 cc for a pressure difference or 73 mm or one of 15.1 cc if the pressure difference were 46 mm, which is not an impossible figure. There appears, therefore, to be some definite relationship between the amount of the leak, the duration of diastole, and the discrepancy between the two ventricular cavities.

Taking this variation in ventricular capacity into consideration it seems probable that the course of events after the production of a lesion is that at first the left ventricle is distended with much more blood than the right, giving a relative dilatation of the left ventricle, and the x-ray photographs in certain cases with a large leak give evidence in the lengthening of the heart that the actual size of the left ventricle may be above that existing normally, more commonly the left ventricle, if dilated, is not large enough to produce such a measurable lengthening. Any such increase in size of the left ventricle during the early stages must depend on a maintained increase in the internal pressure such as that described by Wiggers (11), since three to four days after the production of a lesion (see dogs 22 and 23, table 4) the left ventricular cavity at autopsy was certainly not larger than the right.

Later hypertrophy develops, as indicated by the increased weight of the heart at autopsy, by an increased L/R ventricular weight ratio, and by x-rays which demonstrate an increase in the total area of the heart shadow (but sometimes only after making allowance for the opposite change resulting from the increased heart rate). The development of such a hypertrophy is apparently in progress within 3 to 7 days, since by this time the heart shadow is increasing (see experiments A and E). The figures of experiment B where the increase in heart size was the largest we have observed, show the greater part of this increase had occurred within four months after the initial operation and within two months after the second, and that after this time only a very gradual change was seen in spite of the animal receiving exercise. The measurement of the degree of hypertrophy is complicated by the slowing of the pulse which seems to parallel the development of hypertrophy. The evidence provided by the L/R ratios, by the casts, and by ventricular walls which appear of at least a normal thickness, suggests that the hypertrophy leads to an increase in the length of the fibers around a larger cavity as well.

as to changes in fiber thickness. A heart seen at autopsy having a thick wall associated with an enlarged cavity should, therefore, be classed as hypertrophied rather than dilated, and may be found in a dog whose activity precludes any possibility of decompensation being present (experiment D). This sequence of events is that suggested by Rosenbach "Wahrend diese letztere einer dauernden Vergrosserung der betreffenden Herzohole, auch wahrend der Systole, entspricht, ist erstere nur der Ausdruck einer starkeren Fullung und deshalb grosseren Ausdehnung der Hohle wahrend der Diastole. Es handelt sich also in dem einen Falle um eine constante, absolute, in dem anderen um eine relative Erweiterung, der ersteren geht die Hypertrophe voran, der letzteren folgt sie." There is absolutely no indication that infection must precede hypertrophy.

The relationship of the heart size changes to those observed in electrocardiograms has already been discussed, some change was always in evidence though we agree with Herrmann that the changes attributed to left ventricular preponderance in man are not observed. The increased duration of the QRS group to which attention has been drawn could be accounted for readily by the increased distance the impulses have to travel, whether this increase depends on the larger cavity and a lengthening of the conducting tissue, or on a change in the thickness of the muscle wall.

The figures that have been given for the duration of systole show that this is longer than normal. This change as well as the increase in pulse rate would act to shorten the diastolic period during which regurgitation could occur. Support is, therefore, given to the established clinical belief that a moderately rapid pulse is of benefit in aortic regurgitation, and, however fit, none of our animals have ever developed a really slow pulse. The results are also in agreement with those which Allan (32) has recently obtained in a circulation schema. When failure occurs (experiments E and F) the duration of systole was still above normal, but was found to be decreasing from the levels that had been maintained previously. Even though some electrocardiograms were taken on animals within a few hours of death, the heart failure could not be predicted from the absolute value of the duration of systole observed, though it was indicated by a decreasing systole/cycle ratio (19).

Some effect of the operation on the auricle was suggested by an immediate and marked increase in both potential and duration of the

P wave, further evidence of this was seen at autopsy in thickening of the left auricular wall, which was often quite definite and sometimes the wall of the right ventricle also seemed above normal thickness. No other evidence of back pressure was observed except in experiment C. In general the changes observed agree with the theories advanced by Cohnheim and his pupil Rosenbach and by Marey, and we can only claim to have supported their evidence with data obtained by recent methods which were not available to them.

#### CONCLUSIONS

1 Chronic aortic regurgitation produced in dogs is followed immediately by a diminution in the size of the heart shadow, though later this shows a gradual increase.

2 The pulse rate is raised immediately as the result of the lesion, but the change is rarely sufficient to account completely for the diminution observed in the heart shadow. Later the pulse rate slows but not sufficiently to account for the increase in the heart shadow. The pulse rate never returns to its previous low level, and however fit the animal becomes, a relatively fast pulse appears to be advantageous.

3 The increase in heart size commences probably within the first week and continues rapidly for a period of 1 to 4 months, from 4 months up to 1 year the change is still detectable, but is quite gradual.

4 Deductions of hypertrophy from measurements of heart size by x-ray are much more accurate if allowance is made for the effect of changes in pulse rate. If this is not done only the grossest changes can be detected, and heart shadows below the normal value may be obtained from hearts, which at autopsy can be shown to be hypertrophied by abnormality of the ratios of left to right ventricle and of the heart weight to body weight.

5 The hypertrophy of the left ventricle is associated with an increase in cavity, which is often more obvious than the thickening of the wall.

6 An initial lengthening of the heart shadow is often noted and attention is drawn to the possibility of the left ventricular cavity being increased initially, even though the total heart shadow was decreased.

7 Hypertrophy does not depend on infection.

8 The electrocardiographic records show consistent changes. The potential of any of the waves, except S, may be increased in any

lead, but in particular the Q wave may be much increased, and the S wave is usually decreased in all three leads. The QRS group may become of longer duration, and the lengthening is on the average proportional to the increase in heart size. Systole measured mechanically or electrically is of greater duration than normal, but this lengthening is reduced when failure supervenes. The electrocardiographic changes associated with left ventricular preponderance in man are never seen in our dogs, even when a considerable hypertrophy is known to be present. Both the changes observed in the character of the waves with hypertrophy and the alterations of the same type but of less degree sometimes seen immediately after operation, can possibly be explained as a result of a rotation of the heart.

9 The total lack of symptoms in most cases (except when infection was present) is astonishing, and the ability to do work is often very great, though probably lessened as the result of the operation.

We should like to express our thanks to Dr R T Grant for his examination of the pathological material, and also to Dr J A Eyster\* for helpful criticism at the commencement of the work.

#### BIBLIOGRAPHY

- 1 Cohnheim, J *Allgemeine Pathologie* Berlin, 1877
- 2 Rosenbach, O *Arch f Exp Path and Pharm*, 1878 ix 1 Ueber Artificielle Herzkloppenfehler
- 3 Marey, E J *Circulation du Sang* Paris 1881, p 674
- 4 Tangl, F *Virchow's Arch f Path Anat.*, 1889, cxvi 432 Ueber die Hypertrophie und das Physiologische Wachstum des Herzens
- 5 Hasensfeld and Romberg *Arch f Exp Path u Pharm* 1897, 39 333 Ueber die Reservekraft des Hypertrophischen Herzmuskels und die Bedeutung der Diastolischen Erweiterungsfähigkeit des Herzens
- 6 Stewart H A *Arch Int Med* 1908, 1, 102 Experimental and Clinical Investigation of the Pulse and Blood Pressure Changes in Aortic Insufficiency
- 7 Stewart, H A *Jour Exp Med*, 1911, xii, 187 An Experimental Contribution to the Study of Cardiac Hypertrophy

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\* Somewhat similar observations have been made by Dr Eyster and his co-workers both on aortic stenosis and regurgitation, and their experiments have been carried on simultaneously with ours. We are much indebted to them for an exchange of manuscripts and except for accidental causes the two papers would have appeared together. Their results are in some respects different from ours particularly in that they noted an early cardiac enlargement following operation and the cause of this discrepancy is not clear. Their paper will appear at an early date.

- 8 Stewart, G N Amer Jour Physiol , 1921, lviii, 45 Possible Relations of the Weight of the Lungs and other Organs to Body-Weight and Surface Area (in Dogs)
- 9 MacCallum, W G Johns Hopkins Hosp Bull , 1906, vii, 251 On the Teaching of Pathological Physiology, and Johns Hopkins Hosp Bull , 1911, xxii, 197 The Changes in the Circulation in Aortic Insufficiency
- 10 Wiggers, C J Arch Int Med , 1915, vii, 132
- 11 Wiggers, C J Circulation in Health and Disease Second ed , Philadelphia, 1924
- 12 Sherrington, C Mammalian Physiol Clarendon Press, 1919, p 60
- 13 Straub, H Deut Med Wchnschr , 1919, xlv, 676 Ueber Herzerweiterung
- 14 Sands, J Quart Jour Exp Physiol (Supp Vol), 1923, p 215 Chronic Aortic Regurgitation in Dogs
- 15 Bazett, H C Amer Jour Physiol , 1925, lxxii, 201 Further Observations on Experimental Aortic Regurgitation
- 16 Hermann, G R Jour Clin Invest , 1925, 1, 574 Experimental Heart Disease
- 17 Eyster, J A , and Meek, W J Amer Jour Roent , 1920, 1, 471 Instantaneous Radiographs of the Human Heart at Determined Points in the Cardiac Cycle
- 18 Eyster, J A Communication to Amer Physiological Soc , 1926
- 19 Bazett, H C , and Sands J Annals of Clinical Medicine (In Press)
- 20 Kulbs Arch f Exp Path u Pharm , 1906, iv, 288 Experimentelles über Herzmuskel und Arbeit
- 21 Bazett, H C Amer Jour Physiol , 1924, lxx, 550 Factors in the Causation of Differential Blood Pressure
- 22 Keller, L Pfluger's Arch f d ges Physiol , 1922, cxcvii, 443 Über Gipsausgüsse Einiger Saugtierherzen, Zugleich ein Beitrag zur Schlagvolumfrage
- 23 Lewis, T Heart, 1924, ii, 151 Studies of Capillary Pulsation
- 24 Meek, W J Amer Jour Physiol , 1925, lxxii, 228 The Effect of Changes in Position of the Heart on the Q R S Complex of the Electrocardiogram
- 25 Mines, G R Jour Physiol , 1913, xlvi, 349 On Dynamic Equilibrium in the Heart
- 26 Lewis, T Heart, 1925, xi, 209 Conduction in Mammalian Heart
- 27 Meek, W J Amer Jour Physiol , 1924, lxx, 385 The Effect of Changes in Pulse Rate on Diastolic Heart Size
- 28 Meek, W J , and Wilson, J A Amer Jour Physiol , 1925, lxxii, 228 The Effect of Changes in Position of the Heart on the QRS Complex of the Electrocardiogram
- 29 Joseph, D R Jour Exp Med , 1908, x, 521 The Ratio between the Heart-Weight and Body-Weight in Various Animals
- 30 Anrep, G V , and Segall, H N Jour Physiol , 1926, lxi, 215 The Central and Reflex Regulation of the Heart Rate
- 31 Marshall E K. Amer Jour Physiol , 1925, lxxii, 192 Cardiac Output
- 32 Allan, G A. Heart, 1925, vii, 181 Schema of Circulation with Experiments to Determine Additional Load on Apparatus Produced by Conditions Representing Valvular Lesions

# THE SIGNIFICANCE OF ELECTROCARDIOGRAMS OF LOW VOLTAGE<sup>1</sup>

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The introduction of electrocardiography into the clinical study of heart disease aroused the hope that a method might thereby be available for a quantitative measurement of myocardial power. But unfortunately because of the complicated electrical reaction which is expressed as a resultant in the waves of the electrocardiogram, it became evident that a close correlation was not to be expected between the power of the heart muscle and the amplitude of the electrical deflections of the galvanometer. Moreover it can be shown that fatally damaged hearts are capable of producing wide deviations of the string at a time when they are entirely powerless to provide an effective circulation to the body.

For some years, however, it has been shown by physiologists working with these problems, notably by Einthoven and his pupils, that in animal experiments, in which direct leads can be used and monophasic curves recorded, there is a correlation between the force of muscular contraction, including the heart beat, and the size of the electrical waves produced. In other words the electrical potential difference developed during muscular activity may be an index of the mechanical activity of the muscle.

This observation can not be transferred to the electrocardiogram in man, as recorded from the ordinary axial leads, for the reason that such records are diphasic and are not inscriptions of potential differences in the true electrical axis of the heart. They are in addition relatively crude and compound records of the contraction of a very complicated muscle structure the activity of whose various layers and syncytial connections defies analysis.

<sup>1</sup> Read by title at the meeting of the Society for Clinical Investigation, May, 1926.

From a clinical standpoint, however, certain observed phenomena are deserving of note. It has been found that there are patients who show, electrocardiographically, the condition of "low voltage," which in our study we have taken to mean that the QRS deflections of these patients do not deviate in either direction from the base line more than 5 mm in any of the three customary leads. In other words the difference in electrical potential is not greater than five ten thousandths of a volt.

Such a condition may be taken to mean one of two things, either that the electrical potential difference elaborated during contraction of the heart is actually small, or that the recorded difference is slight because of neutralizing effects within the cardiac muscle. (We are of course assuming a standardized string with a deviation of 1 cm for each millivolt of current.) Either explanation would seem to express an abnormality of contraction in that an actual low voltage means lessened contractile power and a diminished inscribed voltage suggests an asynergy of the units of the muscle mass. It must be realized, however, that such decrease of obvious potential difference may represent conduction defects of the intraventricular block type.

Whatever may be the explanation of this condition it is becoming more evident to those interested in clinical electrocardiography that the finding of records of low voltage is of important diagnostic and prognostic significance. Among those who have called attention to it are Carter (1), Pardee and Master (2), Lutembacher (3), White and Burwell (4), Oppenheimer and Rothschild (5), and Clerc and Bascourret (6). Thacher and White (7), Zondek (8) and others have noted its occurrence in myxedema and cretinism. Oppenheimer and Mann (9) reported seven cases in which it occurred in association with large pericardial or pleuro-pericardial effusions.

#### MATERIAL

With a view to determining the extent of its significance we have studied a series of 57 patients showing the condition. They comprise the total group in which it was observed at the Cardiographic Laboratory of the Massachusetts General Hospital from November 12, 1914, to November 1, 1925.

We have attempted to discover the conditions in which low volt-

age is most commonly found, and to relate it to the clinical aspects of these patients and the progress of their diseases.

### *Etiology*

One is struck immediately with the different incidence of the condition in the two sexes—40 are males and 17 females. This is explained when it is seen that this series falls readily into three main etiological groups—the arteriosclerotic (34 cases), the hypothyroid (10 cases), and the miscellaneous 13 cases.

Of the entire series of 57, 22 are known to be dead (20 males and 2 females), 25 are living (13 males and 12 females), and 10 could not be traced (8 males and 2 females). Of those followed therefore, about 47 per cent are dead.

The arteriosclerotic group is by far the most important. Of these 29 are males and 5 are females. The well known preponderance of arteriosclerosis in males is the obvious explanation of the relative preponderance of males in the entire series. All but 6 of these patients suffered from congestive or anginal failure. Ten of them (all males) had had clinically definite coronary occlusion. Two others had rheumatic heart disease as well as arteriosclerosis. Ten cases had a coexisting hypertension (i.e., a systolic blood pressure of over 160 mm. of mercury was recorded) although probably more had had hypertension but because of the heart failure the pressure had fallen. In 2 cases there was complete auriculo ventricular dissociation, and in 3 cases a complicating neoplastic growth (carcinoma of rectum, prostate and stomach respectively).

In the hypothyroid group 6 had myxedema and 4 cretinism. Of the former 5 were female and 1 was male, of the latter 3 were female and 1 was male. One woman with myxedema had also rheumatic heart disease.

The miscellaneous group was composed of 13 cases in whom low voltage was found. The clinical diagnoses were as follows:

	<i>case /</i>
Syphilitic aortitis and myocardial failure with aortic regurgitation	3
Hypertensive heart disease	2
Rheumatic heart disease	3
Subacute bacterial endocarditis	1
Mediastinal tumor	1

Mediastino-pericarditis	1
Lymphatic leucemia (terminal stage)	1
Unknown etiology	1

*The arteriosclerotic group*

This group comprises 34 cases. Eleven males and 1 female are known to be alive, 3 males and 4 females are untraced. Fifteen, or 44 per cent, are known to be dead and are all males. It is, however, very likely that 6 more, or a total of 61.7 per cent are also dead,

TABLE 1  
*Ages of arteriosclerotic group*

Dead	Living	Untraced
49	53	47
50	54	54
53	55	54*
56	56	64
59	56	65
60	60	71*
60	62	72*
61	62	
62	64	
62	64	
64	65	
68	68	
73		
74		
84		
Total	15	12
		7

\* Almost certainly dead

judging from their clinical conditions at the time of their last observations. In the entire sclerotic group 14 had mainly congestive failure, 10 had mainly anginal failure, 4 suffered from both congestive and anginal failure and 6 did not have either at the time of the record (See table 1).

A total of 9 out of the 11 males known to be alive have been re-electrocardiographed at least once during the course of the entire series. Two have not been reexamined, both have had coronary occlusion—one is working and one is not. The one woman known

to be alive is doing well but has not been electrocardiographed again.

Of the 9 whose electrocardiograms have been repeated, 5 are working and 4 are not. Two have had coronary occlusion—the one in whom the low voltage persisted is not working, the other, who no longer shows low voltage, is working. Seven have not had evident coronary occlusion. Low voltage persists in 4, of whom 2 are working and 2 are not. Low voltage has disappeared in 3, 2 are working and 1 is not.

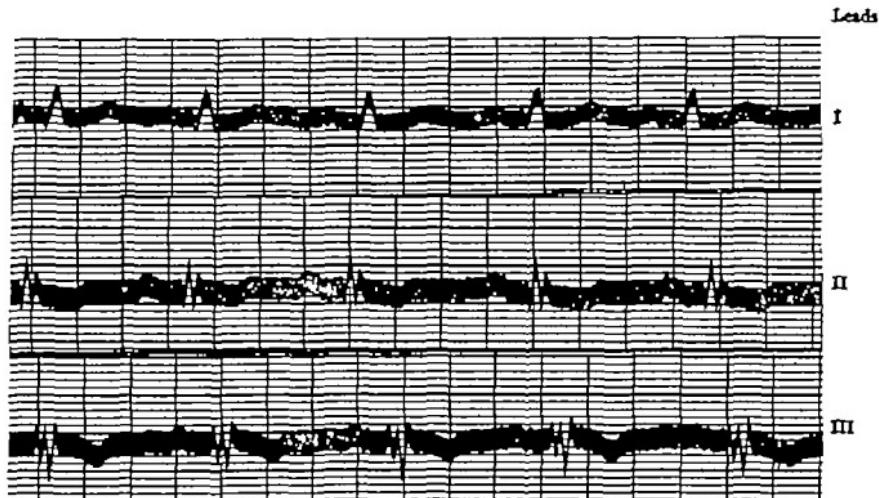


FIG 1 C F W AGE 60 MALE. ARTERIOSCLEROTIC AND HYPERTENSIVE HEART DISEASE MARKED CONGESTIVE FAILURE AND PULSUS ALTERNANS LOW VOLTAGE AND INTRAVENTRICULAR BLOCK

Summarizing the present condition of the 11 males known to be still living we find 6 able to work, and 5 not able to work. Two are working following evident coronary occlusion and 2 are not.

Fifteen patients are known to be dead. Of this group 11 died in less than 6 months after the discovery of low voltage, 13 died in less than a year, and all died in less than 2 years. The clinical prognosis was poor in all of these cases.

The mode of death was as follows

Probable congestive failure	5
Coronary occlusion	4
Post operative (carcinoma)	2
Adams-Stokes attack	1
Angina pectoris	1
Cerebral hemorrhage	1
Unknown	1
Total	<hr/> 15

The quality of heart sounds was noted in 32 out of the total 34 cases of the sclerotic group. Table 2 shows the relation of heart sounds to mortality.

TABLE 2

Heart sounds		Number of patients		
		Dead	Living	Untraced
Good	3	3		
Fair	11	5	5	1
Poor	18	6	7	5
Not described	2	1		1
Total	34	15	12	7

It will be seen that 29 out of 32 in whom the auscultatory findings were noted had some diminution of the sounds. Eleven of those known to be dead had diminished heart sounds, but on the other hand the 3 in whom the heart sounds were of good quality are all dead. Those living all show an abnormal quality of their heart sounds.

Aberration of ventricular complexes, at times of a definite intra-ventricular or bundle-branch block type, was found in more than half of the cases in the sclerotic group. Its importance as a complication is shown in table 3.

In the group of living patients an equal number showed aberration to be present or absent. Twice as many of those known to be dead showed aberration as did not, and of the patients with aberration,

over one-half are known to have died, whereas one-third of those without it are dead.

Five patients had auricular fibrillation, 2 are dead, 2 alive and 1 untraced. Twenty nine did not have fibrillation, 13 are alive, 10 are dead and 6 untraced.

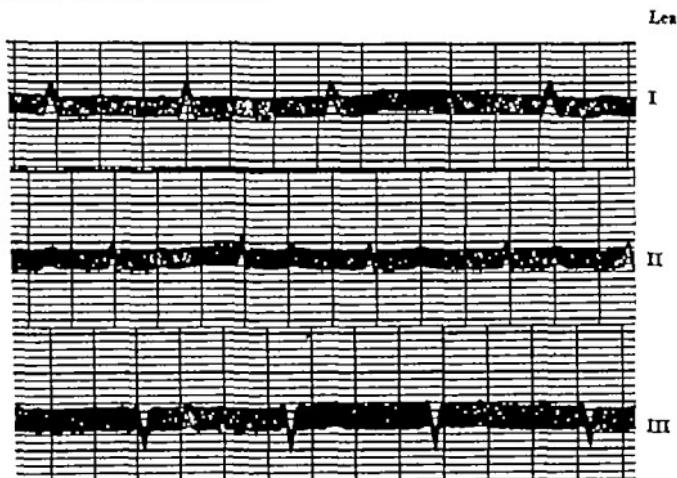


FIG. 2 W M AGE 59 MALE. ARTERIOSCLEROTIC AND HYPERTENSIVE HEART DISEASE AURICULAR FIBRILLATION AND CONGESTIVE FAILURE  
VERY SMALL COMPLEXES WITH SOME ABERRATION

TABLE 3

Condition of patient	Aberration of complexes	
	Present	Absent
Living	6	6
Dead	10	5
Untraced	3	4
Total	19	15

It was thought that there might be some relation between low voltage and the activity of ventricular contraction as seen fluoroscopically. In 4 cases fluoroscopic notes are available. In one, a case of heart block, the independent pulsations of auricles and ventricles were

described. Another heart was said to be beating regularly and the two chambers could be seen to contract normally. The third case

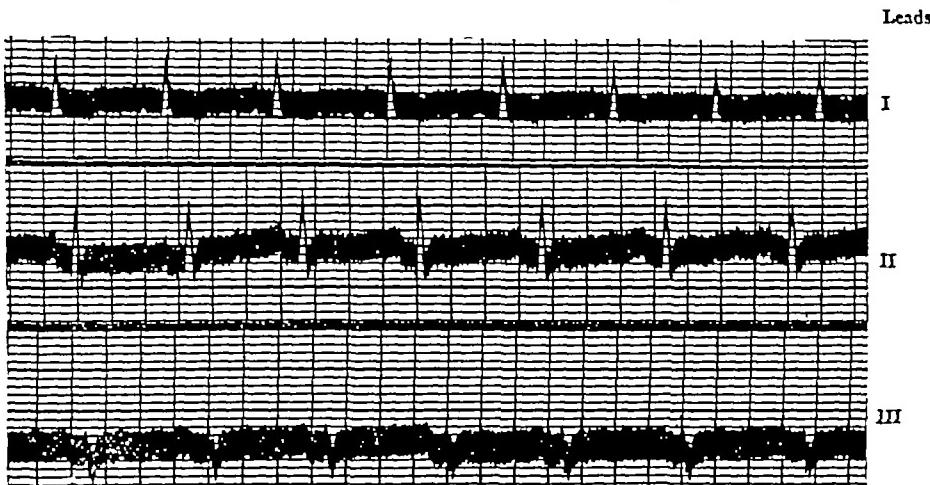


FIG 3 E R AGE 41 FEMALE MYXEDEMA BEFORE TREATMENT

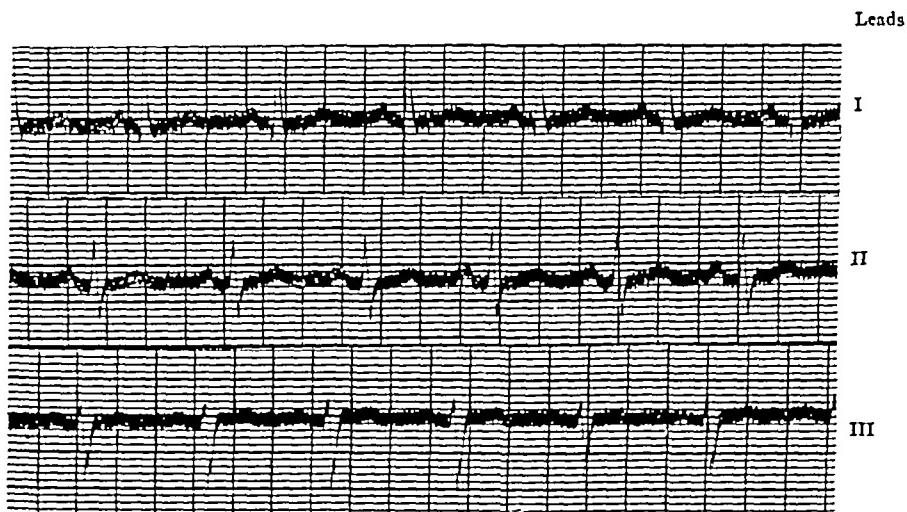


FIG 4 E R SEVEN YEARS LATER, FOLLOWING RETURN TO NORMAL WITH THYROID THERAPY LEFT AXIS DEVIATION

was said to show general enlargement of the heart with limited excursion of the left border, and the last case, the only one now alive, showed the pulsations of the various chambers visible but weak.

Two cases were examined by autopsy. One showed arteriosclerotic thrombotic occlusion of branches of the coronary arteries, chronic

Leads

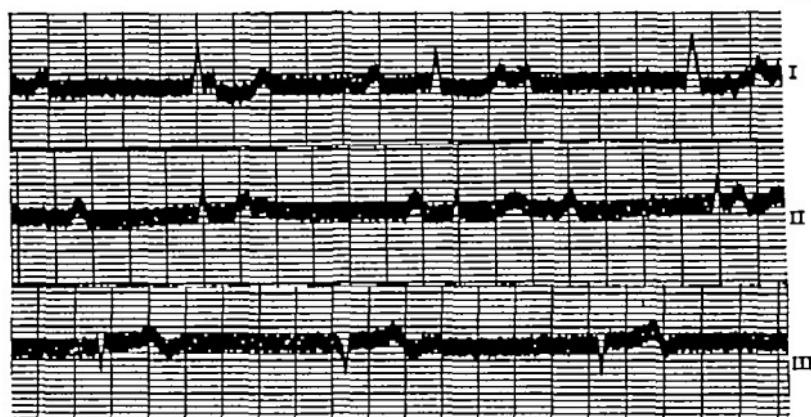


FIG 5 K M AGE 35 NO CLINICAL EVIDENCE OF HEART DISEASE. SMALL COMPLEXES AND COMPLETE HEART BLOCK FOR THE PAST 9 YEARS SOME ABERRATION OF VENTRICULAR COMPLEXES

TABLE 4

	Sex	Age	Diagnosis	Last heard from
1*	M	54	Arteriosclerosis with coronary disease	1918
2*	F	64	Arteriosclerosis Pulmonary embolus. Auricular fibrillation	1922
3	F	25	Adult cretin	1923
4*	M	42	Lytic heart. Septicemia	1924
5*	M	71	Carcinoma of stomach Arteriosclerosis	1924
6*	F	65	Arteriosclerotic heart with congestive failure	1924
7*	F	54	Arteriosclerosis, angina pectoris and congestive failure	1924
8*	F	72	Arteriosclerotic heart disease with marked congestive failure	1924
9	M	32	Chronic mediastino-pericarditis	1925
10	M	47	Coronary occlusion	1925

\* Almost certainly dead.

interstitial myocarditis, myocardial infarction, mural thrombosis of the left ventricle, an area of epicardial softening in the right auricle, slight hemopericardium, thrombosis of a branch of the right pulmonary

artery, infarcts of the lower lobe of the right lung, infarct of the right kidney, chronic pleuritis, and focal organizing pneumonia of the left lung

The other case showed adenocarcinoma of the prostate with extension to neighboring bones, metastasis in retroperitoneal glands, diphtheritic cystitis, miliary abscesses of the kidneys, bronchopneumonia, arteriosclerosis of the coronary arteries, and thrombosis of the left iliac veins

#### *The hypothyroid group*

Hypothyroidism has been known to cause changes in the electrocardiogram which are not necessarily of prognostic importance Thacher and White (7) have recently analyzed a series of such patients showing the effects of treatment

In our group there were 6 cases of myxedema Four of these have been reelectrocardiographed and no longer show low voltage, following thyroid therapy There were also four cretins An increase in the amplitude of QRS waves parallels clinical improvement in these cases

In only 1 case of myxedema was a weakness of heart sounds noted, and in only 1 was there an especially abnormal x-ray description of the heart In this case there was an increase in transverse diameter of the shadow with a suggestion of loss of tone of the muscle

#### *The miscellaneous group*

Thirteen patients of variable etiology were placed in the miscellaneous group Seven of them are known to have died and were obviously suffering from severe myocardial damage or weakness at the time the records were taken, 2 had syphilitic heart disease, and 1 each of rheumatic heart disease with uncontrollable flutter, hypertensive heart disease, subacute bacterial endocarditis, mediastinal tumor, and lymphatic leucemia Two are untraced - 1 with syphilitic heart disease and 1 with mediastino-pericarditis

The 4 who are alive present some problems One patient with mild hypertension and irritable heart has not had another record but is feeling well Another with rheumatic heart disease is reported to be living a 'bed and chair' life The third one with rheumatic heart

disease is no longer in the low voltage group and feels fairly well. The fourth one is a healthy woman of 35 who has had complete heart block and low voltage under observation for 9 years.

In this varied group 7 were said to have had very poor heart sounds 1 case with definite gallop rhythm. Of these 4 are dead 1 is living and 2 are untraced.

Three patients had auricular fibrillation and 1 an ectopic auricular rhythm. Aberrant ventricular complexes occurred in 2.

#### *The untraced cases*

It is fair to assume that most of the cases which we have been unable to trace are dead, as appears from the résumé of diagnoses given in table 4. It is very likely that those patients marked with an asterisk are dead, or 7 out of 10.

#### DISCUSSION

Blood pressure readings are not related in our series to the voltage of the electrocardiogram. Low voltage may be found with high, low, or normal systolic levels. Coronary occlusion, however, in some cases resulted in lowering of the blood pressure coincidental with the development of low voltage. In our series systolic pressures varied from 205 to 90, and diastolic from 120 to 20 or even lower in aortic regurgitation.

The QRS complexes themselves were of various types. The first deflection was either negative or positive. In many the deflections alternated, making two or three small peaks, while in some the string, in a given lead, would move only in one direction from the base line.

The T wave in lead 2 was never over 3 mm. in height and in all but two it was 2 mm. or less. On the original records of the patients it occurred as follows:

*	Upright T <sub>2</sub>	Flat T	Inverted T <sub>2</sub>	Diphasic T <sub>2</sub>
Number of cases	35	15	4	3

Auricular fibrillation was present in 8 cases—3 are alive, 3 are dead, and 2 are untraced but probably dead.

## SUMMARY AND CONCLUSIONS

1 A study has been made of a series of 57 patients seen in the past 11 years at the Massachusetts General Hospital in whom electrocardiograms showed that the QRS deflection was not greater than 5 mm from the base line in any lead. The electrocardiographic and clinical findings are correlated.

2 Low voltage has been found in 44 of these 57 cases (77 per cent) related to two conditions:

- a Myocardial failure from arteriosclerosis—34 cases
- b Hypothyroidism—10 cases

It has also occurred in our series in severely toxic or terminal myocardial states from rheumatic, syphilitic or hypertensive heart disease, mediastino-pericarditis, leucemia, and subacute bacterial endocarditis. In one case it was unexplained and was not incompatible with good health, but occurred in a young woman with complete heart block.

3 The arteriosclerotic group is most important. Only about one-third are known to be alive. 44 per cent are known to be dead and from the condition of those untraced at the time when they were last seen, it is fair to assume that almost two-thirds of the entire group are dead. In 10 cases it accompanied coronary occlusion. All patients known to be dead have died in less than two years after the finding of low voltage, although it is impossible to say how long it may have existed before it was recorded by the electrocardiograph. Those who are alive have all been living less than three years after the low voltage was found. The condition has disappeared in some cases with clinical improvement. The quality of the heart sounds was abnormal in 29 out of 32 patients in which they were described, and is one of the most typical findings in this condition.

4 Low voltage occurred in 10 cases of hypothyroidism. It disappeared in those reacting favorably to thyroid medication.

5 The decrease in amplitude of the QRS complexes to 5 mm in patients in the miscellaneous group in terminal states of heart failure or with severely embarrassed cardiac action is further evidence that this finding is an important sign of myocardial weakness.

6 In the entire series, exclusive of the hypothyroid cases, only 9

patients are able to carry on reasonable activity, but of these, two have had coronary occlusion and one has rheumatic heart disease with mitral stenosis

7 Excluding the temporary effect in hypothyroidism low voltage has never been found, in our experience, in records from normal hearts It is a finding of diagnostic and prognostic importance in forming an opinion of the myocardial ability of any individual

#### BIBLIOGRAPHY

- 1 Carter, E P Arch Int. Med., 1918, xxii, 331 Further Observations on the Aberrant Electrocardiogram Associated with Sclerosis of the Atrioventricular Bundle Branches and Their Terminal Arborizations
- 2 Pardee, H. E B , and Master, A M Jour Amer Med Assoc , 1923, lxxx, 98 Electrocardiograms and Heart Muscle Disease. Also Master, A. M , and Pardee, H. E B Arch Int Med , 1926, xxxvii, 42 The Effect of Heart Muscle Disease on the Electrocardiogram
- 3 Lutembacher, R Bull. Med , 1922, xxxi, 616 The Heart in Hypertension
- 4 White, P D , and Burwell C S Med Clin North Amer , 1921, iv, 1839 The Clinical Significance of Changes in the Form of the Electrocardiogram
- 5 Oppenheimer, B S , and Rothschild, M A Trans Assoc. Amer Physicians 1924, xxxix, 247 The Value of the Electrocardiogram in the Diagnosis and Prognosis of Myocardial Disease.
- 6 Clerc, A. and Bascourret, M Rev de Med Paris, 1924, xli, 587 Les Anomalies des complexes ventriculaires électriques et leur importance pronostique dans le cours de l'insuffisance cardiaque.
- 7 Thacher, C , and White, P D Amer Jour Med Sci., 1926, clxxi, 61 The Electrocardiogram in Myxedema.
- 8 Zondek, H. Munchen med Wchnschr , 1918, xlvi, 1180 Herzbefunde bei endokrinen Erkrankungen
- 9 Oppenheimer, B S and Mann, H. Proc. Soc. for Exper Biol and Med , 1923, xx, 431 An Electrocardiographic Sign in Pericardial Effusion

## KIDNEY FUNCTION IN PNEUMONIA

TABLE I

Number of cases	Hospital number	Day of disease	Temperature	Phthalein output in 2 hours	Urine volume per hour	Blood urea nitro gen gm	Urine urea nitro gen gm	Urea concentra tion index	Remarks
1	5426	3	103	92.6	42.8	0.238	15.72	61.1	
		5	102.2		63.5	0.164	12.74	87.5	
		10	99	87.6	70	0.129	9.79	89.7	Crisis on seventh day
		15	99		63	0.193	9.12	37.5	
2	5422	3	103.8	67.2	67.5	0.253	16.27	75.4	
		4	102.4	83.4	34	0.222	21.37	80.1	
		7	100.3	88.7	53	0.126	9.78	80.7	Crisis on fifth day
		10	100.4	94.3	225*	0.137	2.56		Serum disease
		12	99.6	88.3	75.5	0.099	6.46	81	Serum disease
3	5442	5	104		85	0.165	5.24	45.4	
		9	100.5	82.7	37.5	0.121	8.95	70.2	
		11	99	75.6	28.5	0.132	13.03	81.6	
		19	101	58.9	55	0.116	6.04	59.8	Serum disease
		25	103	80.1	22.2	0.098	7.53	56.1	Serum disease
4	5450	7	102	86.9	58.5	0.149	12.79	87.1	
		10	100	59.1	30	0.150	13.21	64	Crisis on seventh day
		14	100	63.9	55.4	0.108	4.65	42.5	Serum disease
5	5527	4	103.5		168.2	0.214	12.85	97	
		5	102	28	213	0.207	15.00	131.7	
		8	99	68.2	107	0.145	8.52	75.9	Crisis on fifth day
6	5542	6	104	42.1	100	0.136	10.58	103.2	
		14	99	73.9	60	0.172	8.66	51.7	Crisis on seventh day
7	5418	2	103	46.9	0.341	14.58	38.2		
		3	102	55.9	78.3	0.320	12.92	45.5	Died

\* This urine is above the "augmentation limit" and the index cannot be calculated.

TABLE I—Concluded

Number of cases	Hospital number	Day of disease	Temperature F	Phthalate output per cent in 2 hours	Urine volume per hour cc	Blood urea nitrogen per liter	Urine urea nitrogen per liter	Urea concen- tration index	Remark
8	5427	3	103	48.7	95	0.164	6.24	47.2	
		5	103.2	84.4	80	0.381	12.09	36.1	
		9	99.2	92.5	65	0.263	14.64	57.1	Crisis on sixth day
		15	99	83.7	67	0.243	8.40	36	
		18	99	66.1	85	0.156	4.92	37	
		2	99.5		77.5	0.572	11.07	21.5	
9	5436	4	101	65.9	48	0.229	17.94	68.5	Crisis on second day
		6	100.6	49.1	47.5	0.156	12.33	68.8	Mild serum disease
		10	98	79.4	46.5	0.176	13.83	67.6	
		12	98		60	0.162	8.58	51.8	
		4	103.5	32.8	100	0.173	6.95	51.8	
10	5607	9	102	50.3	86	0.405	5.36	15.8	
		14	99	59	71	0.240	7.27	32.9	
		22	99	60.1	28.6	0.152	7.30	33.1	
		4	103.5		95.6	0.279	10.84	44.6	
11	5602	7	102		80	0.303	7.37	25.5	
		12	100	75	50	0.220	16.80	63.4	Crisis on ninth day
		4	103	84.2	62.5	0.332	12.88	39.7	
12	5661	8	99	87.7	42	0.209	14.91	59.8	
		11	99.6	75.2	47.5	0.212	11.98	50.5	
		2	102	60.6	29	0.185	7.89	31.1	Crisis on fourth day
13	5662	6	101	29.5	13	0.127	8.67	33.3	
		9	100	69.4	29	0.110	6.39	42.4	Lysis on fourth day

On the other hand, 4 cases were observed which showed a moderate decrease in the index of concentration. Cases 9 and 10 showed such a result on the day of their crises, in case 9, this was followed by a

relative increase in the index, whereas in case 10, the index failed to reach normal values in subsequent tests. In case 11, there was one subnormal index before the crisis occurred, and in case 13 one sub-

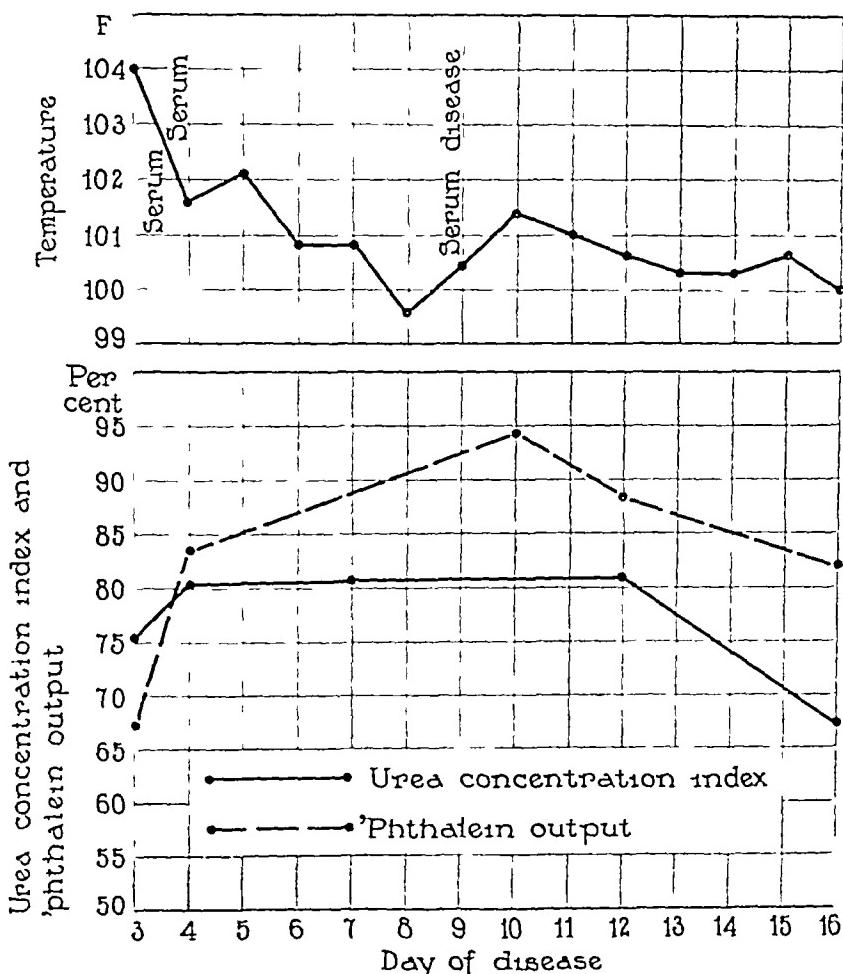


FIG 1 CASE 2 ILLUSTRATING PHASE OF RENAL HYPERFUNCTION AFTER LOBAR PNEUMONIA

normal value before and one after the crisis. Both cases gave normal tests at a later stage of convalescence.

Definitely subnormal phenolsulphonphthalein returns are to be served in cases 5, 6, 8, 10, and 13, one such result being noted in each

case These individual determinations are not in agreement either with the other phenolsulphonphthalein tests in the same patients, nor with the index found on the same day One feels inclined to minimize the value of these determinations as a proof of decreased kidney function in these cases On the whole, however, a moderately good agreement is to be observed between the phenolsulphonphthalein and the index, considering the fact that catheterization was not resorted to in order to obtain complete urine collections

There are two factors which affect the blood urea level which deserve comment The increased protein catabolism of the disease tends to raise the blood urea, and values of over 0.3 grams of urea N per liter are not unusual, even with perfectly normal kidney function On the other hand, the hyperfunctioning kidneys tend to neutralize this tendency, and when hyperfunction occurs, the blood urea tends to be lower than when it does not occur

Whether definite anatomical changes occur in the kidney, with which the increase or decrease in functional ability might be correlated, we are unable to state Albuminuria of slight or moderate degree was present in all the cases during the febrile stage, and it seems reasonable to suppose that some degree of cloudy swelling of the renal epithelium might have been found None of the cases had any history or clinical findings suggestive of coexistent nephritis

The cases have been reviewed to observe whether the type of pneumococcus responsible for the pneumonia bore any relation to the changes found in kidney function No such relation between type of infecting organism and the functional changes is to be observed Six of the cases were diagnosed as Type I, and of these, 5 received antipneumococcus serum Subsequently, large doses of neochunophan were given to 4 of them Four of the cases developed serum disease, manifested by urticaria None of these incidents seem to have influenced kidney function in any way

#### CONCLUSIONS

1 During the course of lobar pneumonia, the kidneys frequently show a period of increased functional ability This period usually begins before the crisis, and lasts until several days after it In a series of 13 cases, the hyperfunction was shown by an increased urea

TABLE I

NUM NTR	NAME	PACT	SEX	AGE	STAGE OF SYPHILIS	BLOOD WnR	SPINAL FLUID	DURATION OF ARTHRITIS	JOINTS INVOLVED		OTHER CLINICAL SIGNS OF SYPHILIS
									Pos	Neg	
1	L D	B	F	23	Early?				?	3 weeks	Temporo mandib , sterno- clavic , acromio-clavic , knees, L ankle
2	J S	B	M	21	Early				Pos	?	Shoulders, elbows, wrists, knees, metacarpal-phalan- geals
3	O W	B	M	55	Early				Pos	3 weeks	Knees
4	R P	B	M	38	Late				Pos	6 years	Right knee
5	J M	B	M	28	Late				Pos	4 months	Left knee
6	B D	B	F	45	Late				Pos	?	Right knee, shoulders
7	N H	B	M	52	Late				Pos	5 weeks	7 months
8	O R	W	M	22	Late congen				?	2 weeks	Right knee, right toe
9	S O	W	M	65	Tubes dor- salis				Pos	10 months	Temporo mandib r elbow, knees
10	L G	B	M	65	Tubes dor- salis				Pos	13 months	Right knee
									Pos	10 months	Right knee
											Signs of tabes dor
											Signs of tabes dor

tests. Several patients with arthritis and syphilis came under observation during this period but had to be excluded because there was insufficient fluid to warrant exploratory puncture. The essential clinical data in regard to these cases are presented in Table I, from which it is seen that the patients were for the most part negroes. There were eight males and two females. They were all of adult age.

*Duration of syphilis.* This information was sought by careful questioning and, in so far as it was possible to judge by the answers of the patient, his age, and the character of the syphilitic lesion present, the stage of the infection has been set down as early or late, it being understood that by "early syphilis" is meant an infection of three years or less, while by "late syphilis" is understood an infection of more than three years duration. We are well aware that such an arbitrary separation is unsatisfactory but it is necessary to set some period for differentiation. Of the ten patients the infection was probably congenital in one and acquired in nine, and of these last it was of recent origin (less than three years) in three and of more remote origin in the remaining six. Two of these six were tabetics.

*Character of the arthritis.* The arthritis was monarticular in three cases and polyarticular in the remaining seven. In two cases there had been one or more previous attacks of arthritis. The relative involvement of the various joints is shown in table I. The duration of joint symptoms, exclusive of the patients with tabes, was from two weeks to six years. In the two patients with tabes dorsalis the joints presented all the essential characteristics of Charcot joints. The duration of joint symptoms in these cases was 13 months and 10 months, respectively.

#### EXPERIMENTAL

*Source of fluid.* The fluid in each instance was obtained from the knee joint by paracentesis. If both knees were involved the one most recently showing symptoms was selected. Procaine was used as a local anaesthetic and almost no discomfort attended the procedure.

*Examination of fluid.* As soon as possible after removal and before clotting, the fluid was subjected to a series of examinations, as follows:

*Wassermann reaction.* This was performed in the same manner in

which the routine test is performed in this hospital (1) except that occasionally a joint fluid would be found which was hemolytic, when undiluted, but if diluted 1-4 was no longer such and could be used for the test

*Cytology* Unfortunately the total number of white cells per cu mm was not determined in all the cases Differential counts were made with stained preparations or with fresh preparations examined under the dark field This latter method of examination we have found excellent for differentiating the cells and is convenient in that it affords an opportunity to search for treponemes and to make a differential count at the same time In reporting our results we have thought it wise to place the lymphocytes and large mononuclear cells in one group and the polymorphonuclears in another

*Bacteriology Smears*<sup>1</sup> Smears were stained by Gram's method and examined for bacteria, particularly the gonococcus

*Cultures*<sup>1</sup> Ordinary aerobic cultures were made on fluid and solid media and in addition cultures were made for the gonococcus on ascitic fluid dextrose agar under partial oxygen tension

*Dark field* All specimens were subjected to dark field examination and at least 100 fields were examined before dismissing the specimen as negative

*Animal inoculation a* Guinea pig In each case two guinea pigs were inoculated subcutaneously with 2 to 3 cc fluid and observed for at least ninety days following inoculation The object of this procedure was to exclude tuberculous infection, if possible

*b Rabbit* Each specimen of fluid was inoculated into the testes of two normal male rabbits From 2 to 4 cc of fluid was used, depending upon the amount of fluid available and the size of the animal In most instances both testes were inoculated Each rabbit was kept under observation for a period of at least ninety days following inoculation

#### RESULTS

The results of the examination of the fluid of these ten patients with arthritis and syphilis are presented in table 2 For the sake of dis-

<sup>1</sup> This procedure was carried out in the Bacteriological Laboratory of the Medical Clinic, which is under the supervision of Dr H L Amoss, and we wish to express our gratitude for the coöperation of those serving in that laboratory

cussion we have apportioned the cases in three categories, depending upon the amount of evidence to show that the arthritis was actually syphilitic in origin. We have placed the two cases of tabes with Charcot joint in a separate category. The designation of the categories and the basis for apportionment were as follows:

*Category I Cases in which the arthritis was almost certainly syphilitic.* In this category we have placed all those patients (and only those) from whose joint fluid treponemes could be recovered. There were three such cases.

*Category II Cases in which the arthritis was very probably syphilitic.* In this category we have placed those cases in which there were manifestations of syphilis other than a positive Wassermann reaction and in which antisyphilitic treatment was followed by prompt and marked improvement in the arthritis and at the same time by healing of the other syphilitic lesions. There were two such cases and in both the syphilitic infection was of long duration. In one of these the arthritis had been present for six years, in the other for four months.

*Category III Cases in which there was no reason to believe that the arthritis was syphilitic in origin.* In this category we have placed those patients who had no manifestations of syphilis other than a positive Wassermann reaction and in whom there was little or no improvement in the arthritis following antisyphilitic treatment. There were three such patients and in all the duration of the infection was long and in one it was probably congenital in origin. In one of these patients (case 7) Gram positive cocci were demonstrable in smears from the joint fluid and the arthritis only cleared up when an infected mastoid was drained.

*Category IV Charcot joints.* In this category we have placed the two patients with tabes dorsalis in whom the clinical picture was that of a Charcot joint. In one of these there was no essential change in the joint after intensive antisyphilitic treatment. The other has not been followed for a sufficiently long period, as yet, to determine the effect of treatment upon the arthritis.

Analysis of table 2 brings out several interesting facts. In the first place it is seen that by rabbit inoculation virulent treponemes were recovered from the joint fluid in three of the ten cases. In all of these the syphilis was recent in origin, and in fact they were the only pa-

TABLE 2  
JOINT FLUID FINDINGS

CATEGORY	CASE NUMBER	STAGE OF SYPHILIS	WaR			Monos per cent	Smear per cent	Cult	Dark field	G P inoc	Rab inoc	EFFECT OF ANTI-SYPHILITIC TREATMENT
				Cells	Polys							
I	1	Early?	Pos	17,600	50	43	Neg	Neg	Neg	++	++	+++†
	2	Early	Pos	21,200	88	12	Neg	Neg	Neg	+-	+	+++†
	3	Early	Pos	24,000	51	49	Neg	Neg	Neg	+-	+	+++†
II	4	Late	Pos	?	46	54	Neg	Neg	Neg	--	--	+++†
	5	Late	Pos	?	24	62	Neg	Neg	Neg	--	--	+++†
III	6	Late	Pos	2,480	89	11	Neg	Neg	Neg	--	--	++†
	7	Late	Pos	1,500	100	0	Gr Pos Cocc.	Neg	Neg	--	--	++†
IV	8	Congen	Pos	?	80	20	Neg	Neg	Neg	--	--	++†
	9	Tubes	Pos	Not done			Neg	Neg	Neg	--	--	0†
	10	Tubes	Pos	?	56	44	Neg	Neg	Neg	--	--	0†

\* Arthritis cleared up after draining of mastoid.  
† h.c.y.

+++ Immediate improvement, restitution of joint to normal  
++ Immediate improvement, joint not completely restored to normal  
++ Moderate improvement.  
+ Slight improvement.  
0 No improvement.

tients with early syphilis and arthritis whom we had an opportunity to study in the manner indicated. They will be considered in greater detail below. Treponemes were not recovered from the joint fluid in any other instance. Dark field examination of the fluid was negative for treponemes in every instance.

Positive Wassermann reactions were obtained with the joint fluid in every instance in which the blood Wassermann reaction was positive. This was to be expected, of course. In one instance (case 9, tabes with Charcot joint) the Wassermann reaction of the joint fluid was positive and that of the blood negative.

Considering the cases in categories I and II as being instances of true syphilitic arthritis, and hence capable of being considered as a group, we find that in four out of five there was a marked tendency of the joint fluid to show a relatively high percentage of lymphocytes and mononuclears, taken together (43 to 62 per cent). The percentage of these cellular elements in the synovial fluid of these cases was, as a rule, higher than that of the corresponding cells in the blood. In case 2, in which the proportion of lymphocytes and mononuclears together in the synovial fluid did not exceed 12 per cent, there is some reason to believe that the arthritis may have been due, in part at least, to an antecedent gonococcal urethritis, although no gonococci were recovered from the joint fluid.

Of the diagnostic significance of this finding of relatively high percentage of lymphocytes and mononuclear cells in the synovial fluid we are as yet uncertain. The cases are too few to permit of generalisation on this point, but it is of interest to note that the same tendency was not observed in those cases in which the therapeutic response to antisyphilitic treatment gave no reason to believe that the arthritis was syphilitic in origin. Swift (2) has found a high percentage of polymorphonuclear cells in the joint fluid in cases of acute rheumatic fever, and Labor and Von Balogh (3) found the polymorphonuclear cells predominant in the joint fluid in cases of acute rheumatic fever, gonococcal arthritis and arthritis developing during the course of dysentery, although in the latter group the lymphocytes sometimes were predominant when the inflammatory process was clearing up. Singer (4) found polymorphonuclears predominating in the joint fluid of patients with dysentery and arthritis. Griffon and Abrami (5)

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	3	Early	Pos	24,000	51	49	Neg	Neg	Neg	Neg	Neg	+-	+++†
II	4	Late	Pos	?	46	54	Neg	Neg	Neg	Neg	Neg	--	+++†
	5	Late	Pos	?	24	62	Neg	Neg	Neg	Neg	Neg	--	+++†
III	6	Late	Pos	2,480	89	11	Neg	Neg	Neg	Neg	Neg	--	++†
	7	Late	Pos	1,500	100	0	Gr. Pos Cocci	Neg	Neg	Neg	Neg	--	++†
IV	8	Congen	Pos	?	80	20	Neg	Neg	Neg	Neg	Neg	--	+†
	9	Tabes	Pos	?	Not done		Neg	Neg	Neg	Neg	Neg	--	0†
	10	Tabes	Pos	?	56	44	Neg	Neg	Neg	Neg	Neg	--	0†

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studied the joint fluid obtained from a patient with recurrent secondary syphilis and coincident arthritis which yielded rapidly to mercurial treatment but relapsed following cessation of treatment. The fluid at first showed what the authors called a "polynuclear formula" which later became lymphocytic as the process became sub-acute. Unfortunately no further details of the cytology are given.

There are comparatively few statements in the literature as to the cytology of the joint fluid in cases of syphilitic arthritis and it is not beyond the range of possibility that the relatively high percentage of lymphocytes and mononuclear cells, such as we encountered in the presumably syphilitic joints, may prove to be a constant finding in syphilitic arthritis and help to differentiate it from other types of acute and subacute polyarthritis. Our cases are, of course, too few to warrant any conclusions as yet but they suggest that the point is worthy of further attention.

#### CLINICAL FEATURES

It has seemed desirable, for purposes of record, to present in some detail the clinical histories of the patients in whom there was good evidence for believing that the arthritis was syphilitic in origin, that is to say, those falling in categories I and II.

#### *Category I Joint fluid inoculation positive*

*Case I* L D, Service No 50596. The history of this case has already been published in detail (6) and will be but briefly summarised here. The patient was a colored woman 23 years old. She had been married 4 years and her husband was known to have had syphilis. She had never had arthritis previously. Two months prior to admission she began rather suddenly to have stiffness and pain in the neck, shoulders and knees, and she was obliged to go to bed shortly afterwards. A month later there was sore throat followed by swelling of the glands in the neck.

On examination she was found to have marked swelling of all the superficial lymph nodes of the body which in addition were quite painful. There was pain, tenderness and limitation of motion of the temporo-mandibular, sterno-clavicular, acromio-clavicular, knee and left ankle joints. No joint could be said to be exquisitely tender. Both knees were moderately swollen and fluid was present in each. The liver and spleen were palpable. The blood Wassermann was positive and the blood count revealed an eosinophilia of 10 per cent. The roentgen ray examination was reported as follows (130779) "Examination of the left knee

shows slightly increased amount of fluid in the joint, indicated by riding of the patella, no bone changes or any evidence of involvement of cartilage, no evidence of any periostitis just adjacent to the joint. From an X ray standpoint with the exception of moderate hydrops the examination is negative. There was slight irregular elevation of temperature the maximum attained being 100.5°F.

Rest in bed was not followed by any marked improvement but following the administration of neo-arsphenamine and arsphenamine there was a prompt improvement in the joint symptoms and diminution in size of the lymph nodes. The arthritis disappeared entirely within 3 weeks after the initiation of antisyphilitic treatment leaving entirely normal joints. A lymph node was excised prior to treatment and inoculated into two rabbits. In both of these animals syphilitic orchitis developed. As indicated in table 2 the joint fluid also gave a positive result on inoculation. The patient has been under constant supervision for a period of over two years and there has never been any recurrence of the arthritis.

*Comment.* The case is of particular interest because there were no cutaneous manifestations of syphilis at any time, yet the infection was in all probability fairly recent. The patient was regarded, at first, as having an acute polyarthritis of unknown etiology until the positive Wassermann reaction directed attention to the possibility of syphilis as the cause.

*Case 2* J S Service No 52059. The patient a colored male 21 years of age had never had arthritis prior to the present attack. He had had an acute gonococcal urethritis shortly before the onset of the present trouble. Three weeks prior to admission he began suddenly to have pain in both shoulders followed almost immediately by painful swelling of both knees.

On examination the shoulders, elbows, wrists, knees and metacarpal phalangeal joints were painful on motion and tender on palpation. The right shoulder, both elbows and both knees were most involved, with fluid in the latter, but no joint was exquisitely tender. There was moderate general lymph node enlargement and a bilateral conjunctivitis. There was no urethral discharge but an indurated area could be palpated on the shaft of the penis. Leucocytes, which were 9,100 on admission reached a maximum of 16,000 within a week and the temperature rose as high as 101°. The differential count was normal. The blood Wassermann reaction was positive. The roentgen ray examination was reported as follows (146592) "Examination of the right knee shows an increase in the synovial fluid, indicated by riding of the patella. No changes in the synovial membranes or bones, no evidence of any periostitis. The joint is negative, except for hydrops."

The joint fluid, aspirated 4 days after admission, gave a positive inoculation result in one of two rabbits. There was some improvement under rest in bed and salicylates but certainly not marked. Sixteen days after admission a maculo-papular syphilitide made its appearance. Following the administration of ars-

phenamine the rash quickly disappeared as well as the arthritic symptoms, and the joints were quickly restored to normal. The patient subsequently stopped coming for treatment and following this lapse a neuro recurrence developed.

*Comment* This case is of considerable interest from a diagnostic standpoint because of the possibility of a recent gonococcal infection being involved in the causation of the arthritis. This possibility must be kept in mind, although gonococci were not demonstrable in the conjunctival secretion nor in the joint fluid, whereas treponemes were recovered from the latter. It is of interest that the onset of joint symptoms preceded the occurrence of the syphilitic rash by about five weeks. Wile (7) has reported a case of early syphilitic arthritis in which the joint symptoms preceded the appearance of cutaneous lesions by at least two months.

*Case 3* O. W., Service No. 53251. The patient, a colored male, 55 years of age, unmarried, had had "rheumatism" in the right knee for about a year previous to admission. It was not sufficient to keep him from his work which was that of a fireman on a vessel. Two months and a half prior to admission he acquired pneumonia from which he recovered. While convalescent from this attack and while yet in bed a sore appeared on the penis. (Two weeks prior to the onset of the pneumonia he had been exposed to venereal infection.) Several days after the appearance of the penile sore the glands in the left groin began to swell, attained the size of a hen's egg and then receded somewhat, without suppuration. This was followed by pain and redness in the eyes, photophobia and lacrimation. Three weeks before admission the left knee became painful, hot, and somewhat swollen, then the left ankle and right knee became involved but were not as painful as the left knee.

On examination there was found a bilateral conjunctivitis and iritis, involving the right eye more than the left. The inguinal glands were markedly enlarged and hard, especially on the left, and there was an indurated scar on the prepuce which had all the characteristics of a healed chancre. There was a moderate genu valgum due to deformity of the right knee which was swollen. This swelling involved the lower end of the femur and the soft parts as well. There was crepitus on motion a small amount of fluid in the joint, slight atrophy of the muscles of the thigh and slight limitation of motion. The left knee was swollen and contained a moderate amount of fluid. It was tender and painful on motion but not exquisitely so. No bone changes could be made out and there was no limitation of motion. All other joints were normal. The roentgen-ray examination was reported as follows (156993) "Examination of the left knee shows slight swelling of the synovial membranes. There is apparently no increase in fluid, as the patella is not riding. There are slight spur-like exostoses arising from ends of patella and external edge of left tibial articulation."

' Examination of right knee shows marked swelling of the synovial membranes, marked arthritis of joint, indicated by some destruction of cartilage of femur with formation of fairly marked exostoses from the under surface of patella, and similar but slight exostoses arising from edges of the articulating surface of tibia, also slight periostitis of femur just near where the synovial membrane is attached. This patient's age is 55 years, and the changes seen in the joint itself are those of a hypertrophic arthritis. The only change suggestive of luetic infection is slight periostitis of femur outside the joint.' The blood count showed 8760 cells per cubic millimeter with 7 per cent eosinophiles and 53 per cent neutrophiles. The maximum temperature attained was 100 0°F. A drop of fluid was aspirated from a left inguinal lymph node and after prolonged search with the dark field microscope a single treponeme was found. There was no acute urethritis.

Following antisyphilitic treatment there was a subsidence of the intus and conjunctivitis, and marked improvement in the condition of the knees, although complete restoration to normal was not attained, the right knee still showing changes but very little fluid. Upon discharge from the hospital the patient disappeared from observation.

*Comment* This case must be regarded as one in which there was an antecedent chronic arthritis of moderate degree, more marked on the right than the left, that exhibited an acute exacerbation following syphilitic infection. The changes in the joint as revealed by clinical and roentgenological examination were essentially those of a long standing process involving bone, cartilage and synovial membrane, upon which an acute hydrops had been superimposed. There seems little doubt but that syphilitic infection played a prominent rôle in the exacerbation of the joint symptoms but by no stretch of the imagination can it be conceived of as having been responsible for the changes in the bone and cartilage, which must have occurred long before the patient acquired his syphilis. It is quite conceivable, however, that the pre-existing chronic arthritis acted to create a favorable nidus for the localisation of treponemes in the joint structures with consequent initiation of a syphilitic process, since experimental evidence has been forthcoming to show that an area of inflammatory reaction in the subcutaneous tissues of the rabbit constitutes a favorable place for the inoculation of treponemes (8).

*Summary* In these three cases we are dealing with patients in the early period of syphilitic infection, exhibiting a polyarthritis of moderate severity with slight fever, from whose synovial fluid characteristic and virulent examples of *T. pallidum* could be recovered by

rabbit inoculation In one, the clinical picture was that of an acute process superimposed upon an old (non-syphilitic) arthritis, in the others the acute process involved joints hitherto unaffected There were no significant features, in connection with the joint changes, from either the clinical or the roentgenological point of view, which would serve to distinguish the arthritis presented by these patients from other types of arthritis of the same degree of severity One striking feature was the relatively slight degree of pain and tenderness of the joints and the absence of high fever The polyarthritis in all three patients yielded rapidly to antisyphilitic treatment

*Category II    Marked improvement under antisyphilitic treatment*

The two cases which we have placed in Category II are similar in that they both were examples of monarticular arthritis developing late in the course of syphilis, accompanied by cutaneous gummatous and yielding promptly to arsphenamine therapy They fall into that group of cases of syphilitic arthritis, monarticular in distribution, that have a definite resemblance to tuberculous arthritis, and that have occupied the attention of French writers particularly (9, 10)

*Case 4 R P, Service No G-83694* The patient, a colored male, 38 years of age, had had a single penile lesion 10 years prior to admission and 4 years prior to the onset of the present illness This lesion was self-treated and healed, leaving a scar It was not followed by any of the usual manifestations of early syphilis Six years before admission the right knee joint became swollen and stiff and remained so thereafter It was red and somewhat tender at times, more so about 10 weeks before admission when hard lumps appeared in the region of the knee beneath the skin Four weeks after their appearance these masses softened and discharged their contents by way of sinuses Four years previously there had been a swelling of the left clavicle which had broken through the skin and discharged for a while but healed spontaneously

Examination showed an extensive scar on the shaft of the penis, thickening of the middle portion of the left clavicle with a scar over this area, and a markedly swollen right knee This swelling involved the periarticular and the subcutaneous tissue, especially above the knee, and there was marked riding of the patella There was grating on passive motion of the right knee, which flexed to 90° only, but the joint was not hot nor very tender Above the knee there were several large communicating sinuses in the midst of an area of infiltrated tissue There was considerable ulceration of the skin where these sinuses issued The

general appearance was that of typical subcutaneous gummata which had broken through the skin (See fig 1) No other joints were involved. The roentgen ray examination was reported as follows (121756) "Examination of the knee does not show any increased fluid in the joint Some swelling around joint—probably



FIG 1 RIGHT KNEE OF CASE 4, R. P., BEFORE TREATMENT, SHOWING SWELLING OF JOINT AND SUBCUTANEOUS GUMMATA

tissues external to the synovial membrane There is, however, some destruction of cartilage and formation of new bone on the top surface of the femur, and beginning spur formation along the articulating edge of the tibia. No evidence of any periostitis The changes impress one as being non syphilitic in character, but are those of an infectious arthritis." Fluid from the knee joint yielded negative

results on animal inoculation. It showed 46 per cent polymorphonuclear cells and 54 per cent lymphocytes and mononuclear cells combined.

Under expectant treatment there was no improvement but following the institution of antisyphilitic therapy there was prompt and marked improvement in the knee. The sinuses healed, the swelling largely subsided, pain and tenderness disappeared, and the patient was able to resume his occupation, that of a waiter. This improvement was manifest after two injections of arsphenamine, and was so satisfactory that the patient no longer thought it necessary to return for further treatment and was persuaded to do so only with difficulty. The joint was not completely restored to normal, even after intensive antisyphilitic treatment extending over a period of a year, but the residual alterations were not marked and from the functional standpoint the result was entirely satisfactory.

*Comment.* There can be little doubt from the prompt and remarkable response to antisyphilitic treatment, that syphilitic infection played an important part in the pathogenesis of the arthritis in this patient. Whether or not it is solely responsible for the changes in the architecture of the joint may perhaps be debatable. The possibility should be entertained that the changes may in part have been due to secondary infection, since there were gummatous sinuses leading to the periarticular tissue, but the cultures of the joint fluid were negative. Again there is the possibility that the arthritic process may originally have been due to some other cause and have been complicated by a superimposed late syphilitic process. There is a third possibility, namely that the rather extensive changes in the architecture of the joint, as demonstrated by destruction of cartilage and new bone formation, may have been the result of continuous trauma to a joint that was the seat of a syphilitic process, and not due to the syphilitic infection *per se*. In the experience of one of us (Baetjer) there is little evidence to show that in acquired syphilis the infection involves the cartilage, hence it has seemed wise to raise the question, in this particular case, whether the changes in the architecture of the joint and more particularly the destruction of cartilage is to be attributed to the syphilitic inflammatory processes, or whether some other factor may not also have played a part. These are matters of speculation and cannot be resolved now so far as this particular case is concerned, but the fact remains that antisyphilitic treatment in this patient brought about a really remarkable improvement within a very short

space of time and converted an incapacitating joint into one which functioned almost normally

*Case 5 J M, Service No G 97672* The patient, a colored male, aged 28, had had a penile lesion accompanied by inguinal adenitis 8 years previous to admission. The sore was cauterised by a physician and healed in two weeks without any subsequent secondary manifestations. His wife had one miscarriage, no other pregnancies. Four months prior to admission he sustained an injury to the right elbow and left knee. Both joints became swollen and painful and shortly afterward two or three small ulcers appeared in the region of the elbow. The condition remained practically unchanged during the succeeding 4 months.

On admission there was found an indurated area 8 x 5 cm on the flexor surface of the right elbow, with a punched out ulcer 2 cm in diameter in the center and several smaller ulcers and pin hole openings in the remainder of the lesion, all of which exuded a sticky exudate. There was no tenderness of the ulna and motion of the elbow was unrestricted. It was thought that the joint itself was not involved in the process, which was regarded as a diffuse gummatous lesion. There was swelling of the left knee and of the left pre-patellar bursa with evidence of fluid in the joint; the latter however, was not tender nor hot. Flexion was limited by about 15° and there was no crepitus. The roentgen ray examination was reported as follows (134483) "Examination of the knee does not show any fluid in the joint and no changes in the cartilage or bones. No evidence of any periostitis. From an X-ray standpoint the examination is negative." The fluid obtained from the knee gave entirely negative bacteriological results, both on culture and animal inoculation. It contained 4800 cells per cubic millimeter of which 24 per cent were polymorphonuclears and 62 per cent were lymphocytes and mononuclears. The remaining 14 per cent were unclassified.

Following the administration of arsphenamine there was prompt healing of the gumma of the elbow and an equally prompt return of the knee joint to normal. The improvement was maintained.

*Comment* The case is of interest in that there was a clear history of trauma preceding the onset of the arthritis. Local treatment had not brought about any improvement in the condition within a period of four months but the response to antisyphilitic treatment was prompt and indeed marked. The arthritis of the knee cleared up as rapidly as did the gummatous process in the neighborhood of the elbow.

*Summary* In both of the foregoing cases the arthritis was monoarticular, involved the knee, and the swelling of the joint was rather out of proportion to the other signs of inflammation. Local heat and tenderness were present in both to a relatively slight extent, so that

the disease of the joint resembled in some respects tuberculosis. In both the response to arsphenamine was striking. From the clinical standpoint these two cases present a striking contrast to those in the foregoing category, yet the improvement of the joint under antisyphilitic treatment was so prompt and so marked, and paralleled so closely the healing of the gummata that there can be little doubt but that syphilitic infection played a rôle in the pathogenesis of the arthritis. These cases correspond more or less closely with the picture described years ago by Richet (11). He drew attention to a form of monarticular arthritis occurring in a syphilitic individual late in the course of the disease, characterised by considerable swelling of the joint with relatively slight pain, tenderness and elevation of temperature of the joint itself, and yielding readily to antisyphilitic treatment.

#### *Category III Probably non-syphilitic*

In the cases falling in Category III, there was nothing in the response to arsphenamine treatment to suggest that syphilis was responsible for the arthritis, and animal inoculation was negative. Indeed in one (No. 7), Gram positive cocci were seen in the smear made from the joint fluid and in this case the arthritis subsided promptly when an infected mastoid was drained. None of the cases in this group are deemed worthy of extended comment.

#### *Category IV Charcot joint*

Both of the cases of tabes with Charcot joint yielded negative joint fluids from the bacteriological standpoint, nor was there any immediate significant change in the condition of the joint itself following antisyphilitic treatment. The histories are not of sufficient interest to warrant further details.

### ROENTGENOLOGICAL FINDINGS

Study of the roentgenograms in the five cases (Categories I and II) which we have regarded as examples of syphilitic arthritis has not revealed any new principle which can be utilised in the roentgenological diagnosis of syphilitic arthritis. The X-ray did not disclose any change in the joints in these cases which was common to all and could

be regarded as peculiarly syphilitic. Such changes as were observed were largely those associated with increase in the fluid content of the joint. It is true that in one of the cases bony changes were found but the history of the case showed clearly that these were associated with a pre-existing hypertrophic arthritis of the sort commonly seen in elderly persons.

Failure of these cases to show any roentgenological signs indicative of syphilis is not, however, to be taken as evidence that the roentgen ray may not disclose syphilitic changes in a diseased joint. The X-ray can and does at times reveal changes in joints which strongly suggest the presence of syphilis as a determining factor in the production of the joint abnormality. This is true in acquired syphilis as well as in the inherited type of the disease. It has been the experience of one of us (Baetjer) that in adults hydrops of the knee joint associated with periostitis immediately adjacent to the joint, where there is no history of trauma, is strongly suggestive of syphilis. Moreover in the Charcot joints that develop in patients with syphilitic disease of the central nervous system the joint changes as revealed by the X-ray are pathognomonic of this condition. In these two groups of cases, then, namely those with Charcot joints and those in which there is periostitis associated with hydrops, the X-ray may be expected to be of value in diagnosis. In addition to these two groups, however, there is still a third and probably fairly large group of cases showing hydrops only, without periostitis, and in this group the X-ray does not reveal any changes which may be looked upon as syphilitic in origin. It so happened that all five of our cases which were regarded as examples of syphilitic arthritis fell within this group. What proportion of cases of syphilitic arthritis in general will be found to fall within this group it is impossible to say, but it seems to us essential that the limitations of the roentgenologic method in the diagnosis of syphilitic arthritis be kept clearly in mind.

#### SUMMARY

Ten cases of arthritis occurring in patients with clinical or serologic evidence of syphilis have been studied with reference to (a) abnormalities of the synovial fluid, (b) presence of treponemes or other micro-organisms in the synovial fluid as determined by rabbit and guinea

pig inoculation and by culture, (c) response to antisyphilitic treatment and (d) roentgenologic findings. The cases represented various stages in the course of the infection, including two patients with tabes dorsalis and Charcot joints. From the response to treatment the arthritis was regarded as syphilitic in origin in 5, or one half of the cases. From the synovial fluid of 3 of these 5 patients strains of *Treponema pallidum* virulent for rabbits were obtained by inoculation of animals of that species. In 4 of these 5 the synovial fluid showed a relatively high percentage of lymphocytes and mononuclear cells combined. No roentgenological findings significant of syphilis were encountered in these patients. The clinical findings are reported in detail.

#### BIBLIOGRAPHY

- 1 Keidel, A., and Moore, J. E. Bull Johns Hopkins Hosp., 1923, xxxv, 16  
The Wassermann Reaction in the Johns Hopkins Hospital
- 2 Swift, H. T. Personal communication
- 3 Labor, M., and Von Balogh, E. Wien Klin Wochenschr., 1919, xxii, 535  
Zytologische und Serologische Untersuchungen der Synovia im Besonderen  
bei Akuten Gelenksentzündungen
- 4 Singer, G. Münch Med Wochenschr., 1915, lxii, 183 Erfahrungen aus der  
letzten Dysenterieepidemie
- 5 Griffon, V., and Abrami P. Cited in Annales des Mal Vener., 1907, ii, 55  
L'hydarthrose de la Syphilis Secondaire Étude Cytologique et expé-  
imentale de l'épanchement
- 6 Chesnev, A. M., Kemp, J. E., and Resnik, W. H. Bull Johns Hopkins  
Hosp., 1924, xxx, 235 Syphilitic Arthritis with Eosinophilia Recovery  
of *T. pallidum* from the Synovial Fluid
- 7 Wile, U. J. Jour Cut Dis., 1914, xxxi, 20 Arthropathy in Secondary  
Syphilis
- 8 Chesnev A. M., and Kemp, J. E. Jour Exp Med., 1925, xl, 487 Studies  
in Experimental Syphilis II The influence of a Non-Specific Inflamm-  
atory Reaction Upon the Development of the Chancre
- 9 Pillon M. Les Formes Cliniques de la Syphilis Articulaire Thèse, Lyon,  
1914
- 10 Gouanantz R. Contribution à l'étude des Arthropathies Syphilitiques  
Thèse, Lyon 1914
- 11 Richet Mem de l Acad de Med., 1853, xvii 249

# A STUDY OF THE ACTION OF AMMONIUM CHLORID AND ORGANIC MERCURY COMPOUNDS

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The elimination of urine from the body is not the function of the kidney alone the blood and the tissues are profoundly concerned. The blood may be considered the connecting link between the other two. The concentration in the blood of those substances which eventually appear in the urine must fluctuate with variations in the chemical state of either the tissues or the kidney or both. It is conceivable, therefore, that an absolutely, or apparently, identical chemical disturbance of the blood might result from either chemical changes in the tissues or disturbance of the excretory function of the kidneys. The general problem of the site of initiation of disease characterized by retention of urinary elements is thus complicated by the possibility that fundamentally different diseases may present the same superficial appearance.

The problem became still more intricate with the demonstration that retention of urea does not parallel retention of salt and water. Edema may be accompanied by normal excretion of urea, and normal urea content in the blood, urea may be retained without edema and even without serious anatomic changes in the kidneys, salt may be retained without edema being demonstrable.

The effect of diuretics is similarly difficult to explain. Diuresis can be produced by those which are known to increase blood flow and by those which cannot be shown to have any such primary effect. It is impossible, therefore, to explain diuresis in every instance on the same hypothesis. The newer diuretics, ammonium chlorid and

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novasurol,<sup>2</sup> have been thought to act in a purely chemical way, by affecting the threshold of excretable bodies in the blood, and the acid-base equilibrium in the blood and tissues. Study of the chemical state of the blood cannot be divorced from consideration of the chemical reactions in the tissues. Whether these diuretics act on the tissues, the blood or the kidney is so important a problem that its elucidation will probably go a long way toward settling the whole question of the formation of urine.

Certain of the chemical constituents of the blood, like urea, will be excreted as soon as they appear, they have no threshold. Others, like the inorganic salts and glucose, must attain a fairly definite level before any escapes. This threshold varies in different individuals, and in the same person at different periods, even in health, in certain diseases the threshold is profoundly altered for one or more of them, even for those that normally have no threshold. This disturbance in the threshold of urea or salts may be associated with marked changes in the acid-base equilibrium of the blood and tissues. In acidosis the carbon dioxide combining-power of the plasma is decreased by acid entering the blood, although the hydrogen-ion concentration may be kept normal by the buffer system.

The effect of these newer diuretics on water retained in the interstices of the body is quite remarkable. Here again, if the mechanism of this action can be explained, the cause of the retention will be clear. There is some relation between the excretion of salts and the excretion of water, edema is often associated with chemical changes in the blood. Haldane found that the ingestion of ammonium chlorid by a normal man caused acidosis, as well as increase in urinary excretion, and he attributes this diuresis to the acidosis.

The object of the present study is to determine what chemical changes, especially in the content of salts and their ions, occurred in the blood and urine during the reduction of ascites and edema by the use of ammonium chlorid and novasurol, singly and in combination, and to compare them with the effects produced by the same medication in the normal.

\* Novasurol and salvrgan were used in these experiments. Novasurol is a double salt of sodium oxymercuri-o-chlorphenoxy-acetate with diethylbarbituric acid. Salvrgan is mercuric acetate salicylallylamid-o-acetate of sodium.

## AMMONIUM CHLORID

Haldane's experiment in which he ingested ammonium chlorid in amounts varying from 5 to 55 grams on six occasions resulted in marked acidosis, as indicated by a fall in the alveolar carbon dioxid and in the carbon dioxid capacity of the blood, and an increase in the excretion of acid, ammonia and phosphates. Baird, Douglas and Haldane made similar observations a little later. Gamble, Blackfan and Hamilton noted acidosis, an increase in plasma chlorid, and a decrease in plasma carbon dioxid in children after they had

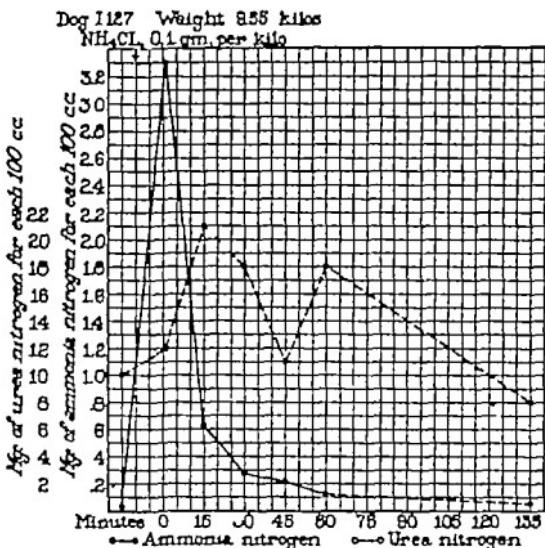


FIG 1 DOG I127 INTRAVENOUS INJECTION OF AMMONIUM CHLORID SHOWING THE RELATION BETWEEN THE AMMONIA AND UREA CONCENTRATION IN THE BLOOD (TABLE 1)

taken 5.8 grams of ammonium chlorid a day for four days. They observed also that there was no appreciable loss of plasma base. They attribute the acidosis, evidenced by the lowering of the plasma carbon dioxid almost wholly to increase of chlorid.

The present studies on ammonium chlorid were carried out on a normal volunteer, on patients, and on dogs. In all cases control periods preceded the giving of ammonium chlorid. The dogs were

TABLE I  
*Effect on normal dogs of ammonium chloride intravenously*



## ACTION OF AMMONIUM CHLORID

TABLE P 2  
*Effect of removal of the liver on the action of ammonium chloride*

Date	Time	Blood				Urine			
		Ammonia nitrogen mgm per cent	Urea nitrogen mgm per cent	Urea nitrogen mgm per cent	Ammonia nitrogen mgm per cent	Urea nitrogen mgm per cent	pH	Volume cc	Potent volumes per cent combining
August 3, 1925	8 25	Blood specimen	Bladder emptied	0 01	6 71	51 8			
	8 30	Animal etherized							
	9 15								
	9 25	Injected 0.25 gram glucose for each kilo- gram in 50 cc saline							
	10 30	Same as above							
	11 25	Catheterized	Glucose injected	0 01	9 20	53 7	3.5	6 3	25 6
	12 30	Injected 0.5 gram glucose for each kilogram in 50 cc saline							
	1 30	Same as above							
	2 25	Catheterized	Glucose injected	0 01	8 05	51 9	90	7 4	26 4
		1 cc for each kilogram, 10 per cent ammonium chloride	Injected						
	3 30	Injected 0.5 gram glucose for each kilogram in 50 cc saline							
	4 30	Same as above							
	5 25	Catheterized							

	6 30	Injected 0.5 gram glucose for each kilogram in 50 cc. saline												
	7 30	Same as above												
	8 25	Catheterized												
August 6 1925	8 30	Blood specimen.	Bladder emptied											
	8 40	Operation	Liver removed at 9:00 a.m.											
	9 15	Injected 0.25 gram glucose for each kilo- gram in 50 cc. saline		5.95										
	11 30	Catheterized	Injected glucose		5.15	5.36	7.0	6.8	42.0	294	448	14.0	98	
	12 25	Injected 0.5 gram glucose for each kilogram in 50 cc. saline												
	1 30	Same as above												
	2 30	Injected 1 cc. for each kilogram, 10 per cent ammonium chlorid		0.08	3.06	49.7	170	7.0	98.6	207	388	32.9	69	
	3 30	Injected 0.5 gram glucose for each kilogram in 50 cc. saline												
	4 30	Same as above												
	5 30	Injected glucose		0.41	2.00	43.9	160	7.0	144.0	32	256	48.0	11	
	6 50	Injected 0.5 gram glucose for each kilogram in 50 cc. saline												
	8 00	Same as above												
	8 30	Blood and urine specimens		0.28	1.90	40.4	260	6.9	91.0	26	338	30.0	9	

given no food during the eighteen hours preceding the experiment and until the study period was completed (usually four hours after the intravenous injection of 10 per cent ammonium chlorid, 0.05 to 0.1 gram (for each kilogram) Human subjects were kept on weighed low-salt, low-fluid diets<sup>3</sup> both before and during the ingestion of ammonium chlorid The average usual dose of ammonium chlorid was 9 grams a day The actual total amount ingested in one case was 364 grams over a continuous period of forty-two days During both control and experimental periods studies of the blood and urine were made

*Blood* Ammonia was determined in the blood of dogs on three occasions, and all the findings were similar The concentration of ammonia nitrogen in the blood rose immediately after the injection, reaching a maximum of 3.3 mgm for each 100 cc in two minutes, but dropped so rapidly that in fourteen minutes it was reduced to 0.61 mgm or one-fifth the maximal value The rapid drop continued for from half an hour to two hours, until the concentration became normal Blood-urea nitrogen did not show a marked change even at the end of two hours in dogs G173 and G185, but with the larger injection in dog I127 (fig 1) it rose from 10 to 21 mgm Plasma chlorids did not change appreciably, the carbon dioxide combining-power of the plasma showed a slight initial drop, which in some cases persisted for two hours Urine collected at intervals of two hours before and after the injection of ammonium chlorid showed an increase in volume in two experiments and a decrease in a third, an increase in hydrogen-ion concentration in all and an increase in the concentration of chlorids in two experiments The ammonium nitrogen did not show any definite constant change, the urea nitrogen concentration remained the same or increased The latter increase is in agreement with previous results (18, 57)

Experiments of short duration give a fair idea of the manner in which the injected salt is distributed in the blood In the case of ammonium chlorid both of the injected ions leave the blood stream

The actual food of these diets contained approximately 800 cc of water, and from 0.6 to 0.8 gram chlorid, from 0.5 to 0.7 gram sodium and from 1.6 to 1.8 grams potassium Complete details in article 'The therapeutic use of diets low in water and mineral content' (31)

TABLE 3  
*Changes in the blood after ingestion of ammonium chlorid*

S.	Age	Sex	Diagnosis	Date	Blood			Dose, per cent of basal power, mgm. per cent, mgm.
					Hemoglobin, gm.	Ammonia, mgm. per cent, per cent, mgm.	Urea nitrogen, mgm. per cent, mgm.	
8	29	M	Normal*	June 26 June 29 June 30 July 1	9.0 0.07 0.05 0.105	378 9.0 11.5 14.0	61 66 54 43	June 26 to July 1, ammonium chlorid 31.5 grams
2	17	M	Subacute glomerular nephritis	January 22 January 24 January 27 January 29 February 2 February 6	11.9	16.5 19.0 21.0 21.0	338 340 371 433	January 29 to February 4, ammonium chlorid 56 grams
3	29	M	Chronic nephrosis	July 14 July 15 July 20 July 24 July 25	13.9	18.0 11.5 0.12 15.0	402 393 372 22	July 15 to 26 ammonium chlorid, 99 grams
4	48	F	Chronic nephrosis	May 28 May 29 June 3		9.0 0.30	354 411	June 2 to 5 ammonium chlorid, 23 grams
5	56	M	Portal cirrhosis	July 23 July 24 July 30 August 4 August 10	11.7	24.0 22.0 17.0 20.0 11.0	48 46 22 47 37	July 26 to August 9, ammonium chlorid, 150 grams

\* See footnote 5

with remarkable rapidity, the chlorid so rapidly that a definite increase in plasma chlorids may not be detected two minutes after injection. The ammonia nitrogen shows an increase but becomes normal in from half an hour to two hours.

The rapid decrease in the ammonia with concomitant rise in the urea content of the blood and increased excretion of urea in the urine in dogs I127 and G175 indicates a quick formation of urea from the ammonium ion of the injected salt. An experiment was carried out by Bollman, Mann and Magath (dog A757, table 2) to ascertain whether urea could be formed from ammonium chlorid in a dog whose liver has been removed. The control experiment had been made after the animal's inferior vena cava and portal vein had been ligated. After removal of the liver and the injection of ammonium chlorid, urea formation diminished and the ammonia nitrogen in the blood remained abnormally high for six hours. The result of this experiment indicates that the ammonium ion is not readily synthesized to urea in the dog with its liver removed, quite the contrary to what occurs in the normal dog. This was to be anticipated from the work of Bollman, Mann and Magath.

In human patients after prolonged ingestion of ammonium chlorid practically all showed an increase in blood-urea (table 3). A normal man (Case 8) ingested 31.5 grams of ammonium chlorid in three days (10.5 grams a day) and his blood-urea nitrogen rose from 9 to 14 mgm for each 100 cc. However, one patient (case 5, table 3) having cirrhosis of the liver with ascites showed a gradual decrease in blood-urea even during the continuous ingestion of 150 grams of the salt for fifteen days. This variation in the blood-urea content after ingestion of ammonium chlorid has suggested a possible relationship between the urea content of the blood, and the presence or absence of water available for its elimination. During this period in the same case, there was no marked disturbance in the carbon dioxide capacity of the plasma. The carbon dioxide combining power of the plasma was 39 per cent by volume at the end of fifteen days, having been 41 per cent by volume on the eleventh day and 59 at the beginning of the period. Nevertheless, in most cases the carbon dioxide combining-power of the plasma underwent a decided drop about the fourth or fifth day after the daily ingestion of the usual

amount of the salt, 9 to 10 grams each day (a total of about 40 grams). The plasma chlorid was usually increased. This increase was particularly marked in cases in which the plasma chlorid was low at the beginning of the ingestion of the ammonium chlorid.

In a few cases the ammonia nitrogen content of the blood was determined before and after several days' ingestion of the ammonium chlorid. A demonstrable increase in the blood-ammonia has not been encountered often. Even after the ingestion of 81 grams of ammonium chlorid (9 grams each day) in one case the ammonia nitrogen of the blood was only 0.12 mgm for each 100 cc. A value as high as 0.30 mgm was obtained in a single case. If the ingestion of ammonium chlorid should result in the accumulation of ammonia in the blood the usual methods for the determination of urea would not give the true value of the blood urea. But the small values which have been obtained thus far in man would not produce an appreciable difference in the urea values since even the highest amount, 0.30 mgm of ammonia nitrogen, would be equal to only 0.65 mgm of urea.

In this series no studies on the sodium, potassium, calcium, and magnesium in the serum were made, but a recent study in a single case (case 6, table 4) after the patient had ingested 39 grams of ammonium chlorid showed no change in the sodium in the serum. In a previous experimental study on dogs, the sodium, potassium, calcium, magnesium and chlorids were determined immediately after the intravenous injection of ammonium chlorid, but no change in these ions was noted. Gamble, Blackfan and Hamilton have also shown that ammonium chlorid causes no appreciable change in the amount of fixed base in the plasma.

*Urine.* The ingestion of ammonium chlorid by a normal subject (case 8) and by two patients (cases 6 and 7) produced no change in the volume of the urine, whereas in the other patients (cases 2, 4, and 5) there was a definite diuresis. In the latter the urine nearly doubled in volume and the volume remained large as long as the salt was ingested (table 4). The cases include nephritis, nephrosis with edema, cirrhosis of the liver with ascites and edema, and endocarditis with ascites and edema. During the first period (ingestion of ammonium chlorid) in cases 2, 4 and 5, the excretion of urine equalled

or exceeded the fluid intake. The patient (case 2) lost weight, almost 4 kilos in seven days. During the second period (no ammonium chloride and diet with increased salts and water) the fluid intake exceeded the excretion of urine. The patient gained in weight. The third period was a repetition of the first. The result in this case (case 2) brings out clearly the possible relation of the diet to treatment in cases of edema (table 5), since in our experience the cessation of ammonium chloride ingestion rarely resulted in an immediate gain in weight when the diet was weighed and low in salt and fluid content.

TABLE 5  
*Combined effect of low-salt low-fluid diet and ammonium chlorid*  
Case 2 Subacute glomerular nephritis

Period	Date 1925	Days	Average daily fluid intake cc.	Average daily urine excretion cc.	Change in weight, kgm	Total ammonium chlorid grams	Diet
1	January 29 to February 4	7	1,560	2,315	-3.6	56	Weight, low-salt, low-fluid
2	February 5 to 10	6	2,025	1,258	+1.4	0	Salt-free, not weighed
3	February 11 to 18	8	1,620	2,310	-4.5	64	Weighed, low-salt, low-fluid

In all cases there was a definite increase in the hydrogen-ion concentration of the urine. The greatest change occurred about the fifth day, after the ingestion of approximately 40 grams of ammonium chlorid, although in the normal subject the hydrogen-ion concentration changed from 5.4 to 4.6 on the second day after the ingestion of 21.0 grams of the salt.

The chlorid excreted was increased in all cases, both in concentration and total amount. The other inorganic anions ( $\text{SO}_4^-$  and  $\text{PO}_4^{3-}$ ) however, were very little affected, although usually the phosphates were slightly increased. Of the basic ions the most marked change was observed in sodium and potassium (table 4). In the normal subject there was a great increase in the excretion of potassium while the sodium actually diminished on the first two days. Gamble, Blackfan and Hamilton made the same observation after calcium chlorid had been ingested. When edema was present there was

usually an increase in the sodium and potassium excreted, the increase in sodium being the more marked. Changes in calcium and magnesium were slight and difficult to evaluate. The total amounts excreted were slightly increased. The sum of the basic ions, calculated as cubic centimeters of one tenth normal alkali, always showed a progressive increase after the ingestion of ammonium chloride, indicating that inorganic basic ions are excreted in large amounts in combination with chloride. Ammonia nitrogen, urea nitrogen, and total nitrogen increased in all cases, the increase in the last being accounted for by the nitrogen in the ammonium chloride ingested.

After the ingestion of ammonium chloride a certain amount of the ammonia appears in the urine, the greater proportion, however, is excreted as urea. The hydrochloric acid which is formed causes acidosis, which in turn causes an increase in the excretion of ammonia and of fixed base. Of the fixed base, sodium is as a rule the chief component, but, if a sufficient amount is not available, an increase in the excretion of potassium occurs.

*Basal metabolism.* Determinations of the basal metabolic rate were made on three different mornings while a patient aged forty-eight, with chronic nephritis and edema, was receiving 10 grams of ammonium chloride daily. After he had taken 30 grams the rate was +1, after 40 grams, -5, after 65 grams, 0. Two hours before the last rate was determined the patient had received 5 grams of ammonium chloride. Such results indicate that the ingestion of ammonium chloride by patients with nephritis and edema has no measurable effect on the basal metabolic rate.

#### ORGANIC MERCURY COMPOUNDS<sup>4</sup>

Saxl and Heilig were the first to discover that novasurol caused a marked diuretic response in cases of cardiac edema. They demonstrated a relative and an absolute increase in the chlorides excreted in the urine. Bohn showed that this occurred in rabbits. Nonnenbruch and Mühlung showed that in normal persons novasurol caused an

<sup>4</sup>The organic mercury compounds used in this investigation were novasurol and salyrgan. The former was supplied by the Winthrop Chemical Company, New York, and the latter by Metz and Company, New York.

## The effect of noradrenaline on the blood of normal persons

increase in the chlorids excreted in the urine, but the latter showed that diuresis did not always occur. We ourselves confirmed these observations in two normal subjects and a dog. In further studies we found that there was a relative and absolute increase in the output of sodium. Saxl and Heilig, and Mühlung, emphasized the early appearance of mercury qualitatively in the urine after the administration of novasurol. We have been able to recover quantitatively a large percentage of the mercury injected (48.5 to 85.6) in the urine of the subsequent twenty-four hours.

*Blood* In the present study the normal subjects and patients were under the same control conditions with regard to diet and fluid intake as those in the previous experiments with ammonium chlorid. Analysis of the blood and serum in two normal men before and after the injection of novasurol (table 6) showed no constant changes in the concentration of hemoglobin, chlorid, sodium, potassium, calcium or phosphates. The carbon dioxid combining power of the plasma remained within normal limits. Nonnenbruch in an experiment conducted under similar control conditions noted no variation in the chlorid concentration, but a slight rise in the protein content during diuresis. No significant changes in the rest nitrogen, uric acid or creatinin were noted by Mühlung.

Studies of the blood in three cases of ascites similarly failed to show any constant changes (table 7). These inconstant findings are essentially in agreement with previous studies reported by Saxl and Heilig, Mühlung, and Bleyer. Crawford and McIntosh report a fall in plasma chlorid for from three and a half to four and a half hours after the injection of novasurol with a later slow rise, also a fall in serum protein in the first one and a half hours after the injection in two cases of edema of cardiac origin. That such early changes occur invariably has still to be corroborated by further work. The constancy of the normal values for the carbon dioxid combining power of the plasma in our cases is worthy of note.

*Urine* An experiment was made on a normal dog (dog G619) to ascertain the effect of novasurol on the urine (table 8). The animal did not receive food or water during the experiment which lasted twenty-four hours. The time was divided into three eight-hour periods, and a specimen of urine was obtained by catheter at the end

The effect of nutrient on the blood in cases of arrears

TABLE 8  
*The effect of novacain on the urine of a normal dog*

Sex	Age	Date	Hour	Volume cc.	pH	Chlorid		Sodium	Total base alkaline base	Phosphorus	Ammonia nitrogen	Urea nitrogen	Total nitrogen, grams
						Grams, per cent, per cc.							
M	897	July 21 1925	7 30 a.m.	20	7.2	0.42	0.084	0.595	0.119	500	100	0.218	0.058
F	897		3 30 p.m.	195	6.8	0.93	1.810	0.420	0.819	495	965	0.038	0.074
			11 30 p.m.	35	6.2	0.19	0.067	0.107	0.037	225	79	0.260	0.091

Injected 0.4 cc. novacain 0.01 cc. for each kilogram

TABLE 9

*The effect of novacain on the urine of normal persons*

Sex	Age	Date	Volume cc.	pH	Chlorid		Sodium	Potassium	Total bicarbonate base	Phos- phorus	Inorganic ammonium	Urea nitrogen	Total nitrogen, grams	
					Grams, per cent, per cc.									
M	29	June 25 1925	450	5.4	0.20	0.86	0.08	0.350	0.291	33.0	0.40	0.03	0.12	148
M	29	June 26, 1925†	1250	5.2	0.44	5.70	0.16	1.960	17.2	20.0	0.02	0.21	0.01	19
			400	5.4	0.02	0.07	0.02	0.10	0.291	13.0	0.03	0.07	0.02	104
M	28	December 4 1924	1000	6.2	0.24	2.40	0.16	1.600	30.3	0.00	—	—	—	416
M	28	December 5 1924†	900‡	5.4	0.58	5.18	0.32	2.840	33.2	99	—	—	—	—
M	28	December 9 1924	7204	0.34	2.23	0.19	1.410	20.1	50	—	—	—	—	0.76
			7255							2.01	1.56	1.07	1.07	0.35

See footnote 5

† Two cubic centimeters of novacain injected.

‡ Divided urine collections show day diarrhea.

§ Divided urine collections show no diarrhea.

of each period Novasurol was injected at the beginning of the second period. Thus, we had a control period of urine excretion before and after the exhibition of novasurol. Following the injection of novasurol marked diuresis occurred, accompanied by a high concentration of chlorid, sodium and total inorganic fixed base in the urine which was maintained, resulting in a striking increase in their total excretion. A moderate rise in the output of nitrogen urea and ammonia with little change in the amount of phosphorus or in the hydrogen-ion concentration also occurred. The noteworthy urinary findings in the third period were a decrease in the volume of the urine and in the output of chlorids, sodium and total inorganic base, even below that of the first control period. The phosphorus excreted was slightly increased while the urea and total nitrogen approximated those of the first period.

A similar experiment was carried out over a three-day period in a normal man<sup>5</sup> (case 8 table 9). His diet was as accurately controlled as that for the patients in the series. As in the dog, novasurol caused an increase in the excretion of water, chlorids, sodium, total inorganic base, and total nitrogen. The excretion of potassium and magnesium was increased, but there was no appreciable change in the output of calcium, phosphorus, sulphate, urea or ammonia. The hydrogen-ion concentration was unchanged. On the third day, or post-novasurol period, the excretion of chlorids, sodium and total inorganic base fell below that of the first day. This result corresponded closely to that obtained in the experiment on dog G619. Nonnenbruch's experiment with a dry controlled diet in a normal student gave similar results as to the chlorid. He did not carry out the other analyses. The continuous increase in the excretion of potassium is of note.

The urinary findings of two other normal subjects (cases 9 and 10, table 9) correspond to the results in the two previous experiments except that frank diuresis was not produced and the excretion of potassium remained unaltered. In one normal subject (case 9) the urine excreted during the day was increased, but the twenty-four-hour output approximated that of the previous twenty-four hours.

<sup>5</sup> A healthy physician with the findings of a normal man except for a temporary orthostatic tachycardia.

Bleyer made a similar observation. In case 10 divided specimens failed to show any evidence of diuresis during the entire twenty-four hours. Under like control conditions, Mühlung reported similar results. The urinary excretion of mercury was determined in a normal subject after the injection of 2 cc. of novasurol, 85.6 per cent of the amount injected was recovered in the twenty-four hour specimen of urine.

The urinary findings in seven cases of edema and ascites and one of myxedema following the injection of novasurol are given in table 10. After ten of the thirteen injections frank diuresis occurred. Continuous diuresis, of forty-eight hours, occurred on three occasions in one case (case 11, table 10). Even more prolonged periods of diuresis occurred, but they were infrequent and their exact significance was difficult to determine. In case 13 the volume of urine was not increased, yet there was a distinct rise in the concentration and total output of chlorids, as in the normal subject (case 10, table 9). Two reactions occurred in case 17 (table 10). With the early injections diuresis was produced, but subsequently the volume of urine and output of chlorids were little affected. A similar failure in diuretic action occurred in case 18 (table 10). This failure of novasurol to cause an increase in the excretion of water and chlorids in the urine was associated in both case 17 and case 18 with a concentration of the plasma chlorid of 276 mgm for each 100 cc., well below the normal renal threshold, 336 mgm.

Urinalysis before, during, and after diuresis in these cases gave results closely simulating those in the normal subject (case 8, table 9). There was the marked increase in water, chlorids, sodium and total inorganic base. The excretion of potassium was variable, being usually, but not always, increased. In case 12 (table 10) it was actually decreased after the first injection of novasurol, and after the second injection the output remained unaltered.

The excretion of phosphate was characterized by a decrease in concentration with little gross change in the total output. The excretion of sulphate, estimated in case 14 (table 10) was unchanged. The excretion of urea, ammonia and total nitrogen was inconstant. Changes in the hydrogen-ion concentration were minor in degree. The post-novasurol period showed a decrease in the excretion of

TABLT. 10  
The effect of nosegush on the urine in cases of edema, ascites and myxedema

15	40	M	Portal cirrhosis	April 12 1925	1 200	6 20	23	2 81		Novaurol 2 cc.
				April 13 1925	2 000	6 20	38	7 48		Novaurol, 2 cc.
				April 14 1925	500	6 00	0	87		Novaurol, 2 cc.
				April 15 1925	1 800	6 00	35	6 36		Novaurol, 1 8 cc.
				April 16 1925	800	6 20	06	0 64		Novaurol 2 cc.
16	60	F	Myxedema	November 3 1924	300	5 00	21	0 66		Novaurol, 2 5 cc.
				November 4 1924	1 025	6 60	63	7 30		Novaurol 2 5 cc.
				November 5 1924	500	5 00	26	1 40		Novaurol 2 5 cc.
				November 6 1924	273	4 80	03	0 14		Novaurol 2 5 cc.
17	55	F	Portal cirrhosis	April 19 1925	200	5 20	17	0 35		Novaurol 2 5 cc.
				April 20 1925	1 300	5 20	29	3 82		Novaurol 2 5 cc.
				April 21 1925	250	5 00	18	0 45		Novaurol 2 5 cc.
				April 22 1925	250	5 40	04	0 00		Novaurol 2 5 cc.
				April 23 1925	750	6 40	14	1 08		Novaurol 2 5 cc.
				April 24 1925	500	5 40	05	0 14		Novaurol 2 5 cc.
18	52	M.	Bent's disease	July 19 1925	950	0 01	0	0 00		Novaurol 2 cc.
				July 20 1925	1 100	0 03	0	0 33		Novaurol 2 cc.
				July 21 1925	1 200	0 02	0	0 24		Novaurol 2 cc.

TABLE II  
*The effect of novarsitol alone and combined novarsitol and ammonium chlorid on the urine*

No.	Date	Diagnosis	Volume cc	pH	Chlorid		Doseage
					Grams per cent	T <sub>95°</sub> grams	
5 29 VI	June 25, 1925	Normal	450	5.4	0.20	0.86	Novarsitol, 2 cc
	June 26, 1925		1,250	5.2	0.44	5.70	
	June 27, 1925		100	5.4	0.02	0.08	
	July 1, 1925		550	4.6	0.68	3.76	
	July 2, 1925		2,100	4.6	0.55	11.47	
	July 3, 1925		550	5.0	0.22	1.22	
6 22 I	March 12, 1925	Chronic nephrosis	650	6.8	0.12	2.33	Novarsitol, 0.5 cc
	March 13, 1925		850	6.4	0.10	2.45	Novarsitol, 1.0 cc
	March 14, 1925		150	6.2	0.73	3.29	Novarsitol, 1.0 cc
	March 15, 1925		450	6.0	0.80	6.03	Novarsitol, 1.0 cc
	March 16, 1925		600	5.8	0.87	5.36	Novarsitol, 1.0 cc
	March 17, 1925		700	5.8	0.66	4.62	Novarsitol, 1.0 cc
	March 18, 1925		800	6.8	0.85	6.82	Novarsitol, 1.0 cc
	March 19, 1925		550	5.6	0.25	5.15	Novarsitol, 1.0 cc
	March 20, 1925		600	5.4	0.92	5.51	Novarsitol, 1.0 cc
	March 21, 1925		1,050	5.8	0.56	5.88	Novarsitol, 1.0 cc
11 17 VI	March 22, 1925		1,800	5.8	0.73	13.08	Novarsitol, 1.0 cc
	March 23, 1925		550	5.8	0.84	4.62	Novarsitol, 1.0 cc
11 17 VI	November 18, 1924	Portal cirrhosis	300	5.8	0.13	0.40	Novarsitol, 2 cc
	November 19, 1924		575	5.6	0.40	2.28	Novarsitol, 2 cc

19	52	M	Portal cirrhosis*		November 20, 1924 November 24, 1924 November 25, 1924	350 700 3,200	5 6 6 8 5 2	0 05 0 28 0 56	0 17 1 93 18 05	
					May 5, 1925 May 6, 1925 May 7, 1925 May 10, 1925 May 11, 1925 May 12, 1925 May 14, 1925 May 15, 1925	1,050 1,150 1,100 800 3,950 1,050 800 1,900	6 2 6 6 5 8 4 8 5 0 5 0 5 6 4 8	0 15 0 15 0 41 0 78 0 62 0 62 0 64 0 60	1 50 1 74 4 50 4 32 62 6 48 5 14 11 40	
									Novasurol, 2 cc. Novasurol, 2 cc.	
20	48	M	Portal cirrhosis		January 10, 1925 January 11, 1925 January 12, 1925 January 14, 1925 January 15, 1925 January 16, 1925 January 17, 1925 January 18, 1925 January 19, 1925	400 800 400 850 1,100 850 1,850 750 2,200	6 2 5 6 5 6 5 8 5 6 5 4 5 8 5 8 6 0	0 51 0 64 0 32 0 87 0 79 0 83 0 72 0 70 0 70	1 98 5 08 1 28 7 40 8 70 7 09 13 32 5 28 15 30	Novasurol, 1 cc. Novasurol, 24 grams Novasurol, 0 5 cc. Novasurol, 0 75 cc. chlorid, 33 grams Novasurol, 1 cc. chlorid, 51 grams

\* See footnote 5

chlorids, sodium and total fixed base, as in the normal control experiments

#### COMBINED EFFECTS OF AMMONIUM CHLORID AND ORGANIC MERCURY COMPOUNDS

By the extensive use of any new therapeutic agent one soon learns to limit its use to certain specific conditions. Ammonium chlorid produced striking diuresis in our early cases of edema. We then

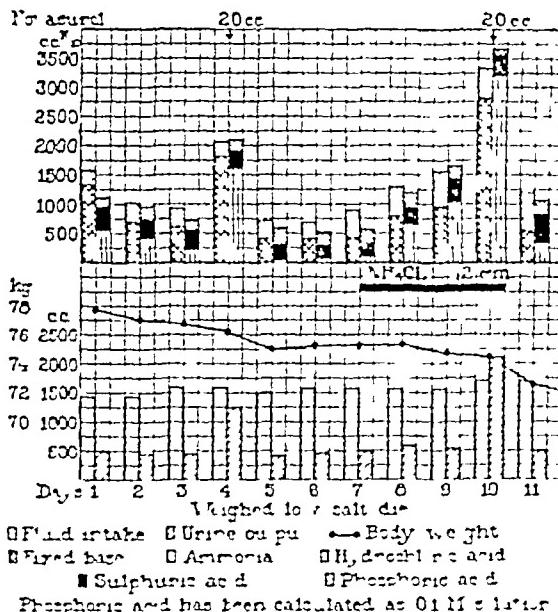


FIG. 2 RELATIVE DIURETIC EFFECT OF NOVASUPOL ALONE, AND NOVASUPOL PLUS AMMONIUM CHLORID, ALSO THE SUM OF THE INORGANIC ACID AND INORGANIC BASIC ION'S PLUS AMMONIA IN THE URINE CALCULATED AS ONE-TENTH NORMAL CUPRIC CENTIMETERS, ACID OF ALKALI (CASE 8, TABLES 3, 4, 9, 11, 12, 13 AND 14)

began to have cases in which the salt failed to act. Similarly novasupol was in our first cases very effective in reducing edema and recites. Subsequently it also failed, in certain cases, to produce the desired diuresis. These facts led us to a trial of their combined diuretic effects in cases in which one or the other had previously failed or produced only temporary slight diuresis. The therapeutic results

of ammonium chlorid and novasurol in combination has, in many cases, been really remarkable, the loss of weight due to removal of previously retained fluid amounting to as much as 70 pounds (31.8 kgm) in from five to six weeks.

Comparison of the results obtained before and after the use of ammonium chlorid and novasurol combined are well demonstrated in table 11. In the normal control, case 8, the volume of urine is distinctly greater, 2100 cc., after the combined drugs than after novasurol alone, 1250 cc (fig 2). In case 13 novasurol produced no diuresis on November 19, whereas with the combined drugs the output of urine six days later reached 3200 cc.

Such results established the efficacy of this form of treatment on a sound basis. We have since made numerous studies directed chiefly toward elucidating further the problem of the action of these substances. Interesting changes in the acid base equilibrium, water balance, the concentration of chlorid and other inorganic ions, and in the formation of urea and ammonia have occurred which are of biologic significance and may be useful in throwing light on other allied problems.

*Blood.* Studies of blood concentration were carried out in detail in case 21 (table 12). The patient received the usual constant diet, low in water and salt (3 grams of ammonium chlorid daily as a substitute for sodium chlorid), throughout his stay of five weeks. Novasurol was given on seven days, with marked diuresis (a maximum of 2900 cc.). During four of the periods of diuresis the hemoglobin increased in concentration, and in one the plasma protein increased in like manner. The plasma water showed some minor hourly variations which were not constant. The plasma chlorid remained unaltered during one period, but fell distinctly at the end of the twenty four hours in another. The carbon dioxid combining power of the plasma deviated from the normal once with a moderate fall. No changes of significance were observed in the concentration of sodium, potassium, calcium and phosphate in the serum. In a normal subject and in a patient with portal cirrhosis, who were receiving 10 grams of ammonium chlorid daily (cases 8 and 22, table 12) the chlorid concentration fell slightly in one but remained constant in the other.

TAN F 12

Dose	Sensit.	Mean per cent	Dose			
			1/2	1	2	3
1/2	Normal	394	9	30	1 m	
1/2	Normal	July 1, 1925	9	30	1 m	
1/2	Normal	July 2, 1925	9	30	1 m	381
1/2	Normal	July 3, 1925	9	30	1 m	390
1/2	Normal	July 4, 1925	9	30	1 m	378
1/2	Bunts' dis cyc, 1 ciles	November 1, 1924	9	00	1 m	358
1/2	Bunts' dis cyc, 1 ciles	November 1, 1924	12	00	noon	351
1/2	Bunts' dis cyc, 1 ciles	November 1, 1924	3	00	p m	363
1/2	Bunts' dis cyc, 1 ciles	November 1, 1924	6	00	p m	348
1/2	Bunts' dis cyc, 1 ciles	November 1, 1924	9	00	p m	361
1/2	Bunts' dis cyc, 1 ciles	November 11, 1924	9	00	a m	315
1/2	Bunts' dis cyc, 1 ciles	November 11, 1924	9	00	1 m	101
1/2	Bunts' dis cyc, 1 ciles	November 11, 1924	12	00	noon	106
1/2	Bunts' dis cyc, 1 ciles	November 11, 1924	3	00	p m	105
1/2	Bunts' dis cyc, 1 ciles	November 11, 1924	6	00	p m	109
1/2	Bunts' dis cyc, 1 ciles	November 11, 1924	9	00	p m	111
1/2	Bunts' dis cyc, 1 ciles	November 11, 1924	9	00	1 m	117
1/2	Normal	July 29 to July 2,				
1/2	Normal	ammonium chlorid, 1/2 grms				
1/2	Normal	Novitsurol, 2 cc				
1/2	Normal	Novitsurol, 2 cc				
1/2	Normal	Octover 22 to No- vember 29, am- monium chlorid, 1/14 grms, 3 grms drul				
1/2	Normal	Novitsurol, 25 cc				
1/2	Normal	351 16 39 1				

\* See footnote 5

TABLE 13

*The effect of combined ammonium chloride and novarsurol on certain constituents of the blood*

Case	Age	Sex	Diagnosis	Date	Dosage		
					Mlood urea nitrogea mgm per cent	Plasma citrullin, mgm per cent	Plasma carbogen dioxide volumes per cent
8* 29	M	M	Normal	July 2 July 3	11.0 21.9	0.10 0.18	Novarsurol, 2 cc Novarsurol, 12 grams
4 48	F	F	Chronic nephrosis	June 3 June 8 June 9	12.2 18.6	11.1 18.6	June 1 to 7, ammonium chloride, 13 grams June 6, novarsurol, 2 cc
6 22	F	F	Chronic nephrosis	March 12 March 13 March 18 March 23 March 26 April 2	5.1 7.15 7.9 7.0 7.0	378 390 378 384 378	March 13 to 30, ammonium chloride, 156 grams Novarsurol, 2 cc Novarsurol, 2 cc March 22, novarsurol, 2 cc Novarsurol, 2 cc March 31, novarsurol, 2 cc
7 35	M	M	Chronic endocarditis and polycythemia	June 18 June 22 June 25	— 13.0	354	Novarsurol, 2 cc 120 grams Novarsurol, 2 cc Novarsurol, 2 cc
18 52	M	M	Banti's disease	July 20 July 25 July 29 August 1 August 8	18.4 22.0 21.4 19.6	276 312 312 366 354	July 21 to August 20, ammonium chloride, 207 grams Novarsurol, 2 cc Novarsurol, 2 cc Novarsurol, 2 cc August 1, novarsurol, 2 cc

20	48	M	Portal cirrhosis	January 12	9.8	379	54	January 11, novasurol, 2 cc.
				January 14	9.3	397	45	January 13 to 19, ammonium chlorid, 60 grams
				January 19	14.8	404	36	Novasurol, 2 cc.
22	27	F	Portal cirrhosis	May 11	9.8	396	48	May 5 and 7, novasurol, 2 cc
				May 12	7.0	366	58	Novasurol, 2 cc.
				May 13	9.8	378	59	April 29 to May 13, ammonium chlorid, 150 grams
23	52	F	Chronic nephrosis	January 19	6.0	383	50	January 19 to 27 ammonium chlorid, 90 grams
				January 21	10.3			January 23 and 25, novasurol, 0.5 and 1.0 cc. respectively
				January 26	16.3	424	28	
24	18	M	Chronic nephrosis	August 17	6.9	358	70	August 18 to September 22, ammonium chlond, 324 grams
				August 22	12.2	358	51	Salyrgan, 2 cc.
				August 24	14.0	376	46	Salyrgan, 2 cc.
				August 28	14.0	376	43	Salyrgan, 2 cc.
				August 31	9.3	340	45	Salyrgan, 2 cc.
				September 3	14.0	376	48	Salyrgan 2 cc.
				September 7	13.5	400	44	September 7, 10 and 14, salyrgan, 2 cc
				September 17	9.8	364	45	Salyrgan 2 cc.
				September 21	10.4	334	46	Salyrgan 2 cc.
25	26	M	Chronic poly serositis	August 13	6.0	400	68	August 13 to September 23, ammonium chlorid, 364 grams
				August 19	12.6	370	52	August 16, 18, and 21 novasurol, 2 cc.
				August 25	9.3	682	56	Novasurol, 2 cc.
				August 31	11.7	406	37	Novasurol, 2 cc.
				September 4	15.8	430	43	Novasurol 2 cc.
				September 16	14.8	364	61	Novasurol 2 cc.
				September 21	16.6	322	68	Novasurol, 2 cc.

TABLE 13—Continued

Case	Age	Sex	Diagnosis	Date	Dosage	
					Blood urea nitrogen, mgm., per cent	Blood ammonia nitrogen, mgm., per cent
26	60	M	Chronic nephrosis	March 14	12.0	44
				March 18	11.2	54
				March 21	20.0	16
				March 25	20.5	35
27	63	M	Myocardial degeneration, decompensation, portal cirrhosis, chronic nephritis	January 24	26.6	373
				January 25	25.6	357
				January 26		60
						January 22 to 31, ammonium chloride, 90 grams Novtsurof, 1 cc
						March 15 to 21, ammonium chloride, 75 grams Novtsurof, 2 cc
						March 22, novtsurof, 2 cc Novtsurof 2 cc

\* See footnote 5

Since ammonium chlorid alone caused a rise in blood-ammonia, blood-urea and plasma chlorid, and a decrease in carbon dioxid combining power of the plasma, it was of interest to ascertain what the added effect of novasurol would be. The results of such a combined action are given in table 13. In the normal subject (case 8) there was a rise in ammonia and urea but no fluctuation in either the chlorid or carbon dioxid combining power, although both of the latter were abnormal, the chlorid being increased above the normal and the carbon dioxid combining power decreased. The results in cases 4, 18, 23, and 26, the rise in blood urea and plasma-chlorid and the fall in carbon dioxid combining power were identical with those in which ammonium chlorid alone was ingested (table 3). In Cases 24 and 25, there was a moderate rise in blood-urea and chlorid and a moderate early decrease in the carbon dioxid combining power. With the long-continued administration of both substances (more than a month) these blood values tend to approach the normal. Cases 6 and 22 show that a low content of blood urea, an increased content of plasma chlorid, and a normal value for the carbon dioxid combining power can be maintained after continued large doses of ammonium chlorid and novasurol. Normal values for urea, chlorid and carbon dioxid combining power were present in cases 21 and 25 (tables 12 and 13) at some time during the combined form of treatment. It is noteworthy that these normal values were found during periods of active diuresis in cases of long standing marked edema, after long-continued administration of both drugs.

The effectiveness of ammonium chlorid in increasing a subnormal content of plasma chlorid is well illustrated in case 18 (fig. 6, tables 10, 13 and 14). Within eight days the subnormal level of 276 mgm. was raised to normal, 342 mgm., thus making it possible for novasurol to cause a satisfactory diuresis. Dilute hydrochloric acid was substituted for ammonium chlorid in case 30 (table 15). The patient had portal cirrhosis with ascites. This acid caused a fall in blood urea and only slight changes in plasma chlorid and the carbon dioxid combining power of the plasma, yet the subsequent administration of salyrgan caused a large increase in the volume of the urine.

*Urine.* In our experience diuresis was usual, following the satisfactory administration of ammonium chlorid and novasurol. The

TAB  
 The effect of combined ammonium  
 chlor<sub>4</sub> Cr<sub>2</sub>O<sub>7</sub>

Case	Age	Sex	Diagnosis	Date 1925	Blood			Chlorid	Sodium	Potas-	Calci-	Magnes-	Pro-	
					Urea nitrogen mgm per cent	Chlorid, mgm per cent	CO <sub>2</sub> combining power, vol umes per cent							
8*	29	M	Normal	July 1	16.0	384	55	550	4.6	0.68	3.76	0.03	0.15	0.49
				July 2	14.0	384	43	2.100	4.6	0.53	11.47	0.20	4.15	0.13
4	48	I	Chronic nephrosis	July 3	21.6	390	43	500	5.0	0.22	1.22	0.03	0.20	0.24
				June 4	12.0	411	37	900	6.0	0.46	4.03	0.14	1.23	0.12
				June 5				1.200	5.2	0.35	4.25	0.13	1.50	0.14
				June 6				3.100	5.6	0.52	16.00	0.28	8.68	0.06
7	35	M	Chronic encar- ditis, polycy- themia	June 7				1.050	5.4	0.53	5.61	0.26	2.70	0.10
				June 21				300	5.2	0.38	1.15			
				June 22	13.0	366	48	1.750	5.2	0.56	9.27			
				June 23				2.050	4.8	0.56	11.44			
18	52	M	Banti's disease, ascites	June 24				1.000	5.6	0.51	5.01			
				July 31	21.6			700	5.6	0.16	1.13	0.13	0.94	0.11
				August 1	21.0	366	33	2.650	6.0	0.43	11.45	0.13	3.44	0.17
				August 2				2.200	6.2	0.40	8.70	0.10	2.31	0.15
				August 3				1.300	6.4	0.38	4.95	0.16	2.29	0.09
				August 4	17.0	372	46	3.175	6.4	0.41	12.90	0.15	4.73	0.09
				August 5				1.150	6.2	0.36	4.14	0.05	0.53	0.07
20	48	M	Portal cirrhosis	August 6				950	5.6	0.13	1.55	0.03	0.33	0.05
				January 18				750	5.8	0.70	5.28	0.34	2.53	0.15
				January 19	14.3	401	36	2.200	6.0	0.70	15.30	0.39	8.62	0.09
22	27	I	Portal cirrhosis	August 15				850	4.6	0.52	4.34			
				May 10				850	5.6	0.66	5.64	0.13	1.10	0.09
				May 11	9.7	396	48	3.700	5.0	0.61	13.76	0.30	11.19	0.06
24	18	M	Chronic nephrosis	May 12	6.9	366	58	1.550	5.4	0.56	8.82	0.21	3.27	0.10
				September 2				500	5.6	0.69	3.39	0.01	0.03	0.21
				September 3	14.0	376	48	3.700	5.4	0.53	19.50	0.20	7.55	0.05
25	26	M	Polyserositis	September 4				550	5.6	0.48	2.64	0.02	0.14	0.10
				August 16				1.000	6.4	0.61	10.40	0.38	6.43	0.03
				Total				2.025	5.4	0.56	11.47	0.35	7.09	0.01
26	60	M	Chronic nephrosis	August 17				1.950	7.2	0.56	10.99	0.40	7.76	0.01
				August 18				5.675			32.81		21.28	
				August 19	12.4	370	52	5.710	5.4	0.53	30.49	0.24	11.28	0.10
				September 21				715	5.0	0.47	3.34	0.04	0.25	0.06
				September 28				700	5.4	0.17	1.19	0.02	0.13	
27	63	M	Chronic myocar- dial degenera- tion chronic nephritis por- tal cirrhosis	September 29				600	5.8	0.16	0.97	0.01	0.05	
				September 30				500	5.2	0.26	1.32	0.02	0.11	
				October 1	18.0	324	70	400	5.6	0.29	1.15	0.02	0.08	
28	26	F	Polyserositis	March 24				750	5.4	0.34	2.52	0.11	0.84	0.09
				March 25	20.0	420	35	2.800	5.4	0.51	14.88	0.28	7.84	0.03
				March 26				1.300	7.0	0.46	5.94	0.27	3.52	0.04
29	20	F	Polyserositis	January 24	26.0	373	62	1.350	4.6	0.66	9.24	0.39	5.92	0.13
				January 25				2.850	4.4	0.66	19.50	0.44	12.68	0.07
				January 26	24.4	357	60	1.650	4.6	0.46	7.50	0.29	4.62	0.10
30	20	F	Polyserositis	March 1				300	6.4	0.65	1.94	0.07	0.22	0.12
				March 2	12.0	402	48	2.400	6.2	0.64	15.26	0.30	7.32	0.07
				March 3				400	6.0	0.58	2.30	0.06	0.25	0.11

\* See footnote 5

† Full diet not taken

LE 14  
chlorid and novasurol on blood and urine

S. No.	Date	No. of experiments	Magnesium	Phosphorus	Inorganic sulphur as SO <sub>3</sub>	Total inorganic base	Ammonia nitrogen	Urea nitrogen	Total nitrogen, grams	Total inorganic acid, N/10 cc.	Total inorganic base and ammonia, N/10 cc.	Dosage					
												Grams, per cent	Total, grams				
102	1930-12-21	0 02	0 110	0 140	0 77	0 21	1 61	172	946	0 16	0 88	1 44	8 90	11 00	2 200	1 576	June 29 to July 2, ammonium chlorid 42 grams Novasurol 2 cc.
103	1930-12-21	0 01	0 190	0 020	0 46	0 05	1 16	132	2 793	0 03	0 73	1 35	7 25	9 90	3 869	3 317	
104	1930-12-21	0 02	0 110	0 150	0 85	0 33	1 83	100	550	0 17	0 96	1 52	8 36	10 00	1 603	1 232	
105	1930-12-21	0 007	0 060	0 04	0 38	0 03	0 30	83	757	0 11	1 00	2 26	2 34	5 10	1 591	1 465	June 1 to 7 ammonium chlorid, 43 grams Novasurol, 2 cc.
106	1930-12-21	0 005	0 050	0 040	0 54	0 04	0 44	28	1 056	0 05	0 55	0 33	3 92	6 00	1 835	1 695	
107	1930-12-21	0 003	0 100	0 010	0 46	0 03	0 84	139	4 309	0 03	0 84	1 19	5 80	7 00	5 159	4 815	
108	1930-12-21	0 004	0 040	0 042	0 03	0 32	139	1 460	0 06	0 68	0 27	2 84	5 40	2 069	2 209		
								0 110	33	0 13	0 39	135	405	0 17	0 52		655 776
								0 020	35	0 04	66	160	2 800	0 03	0 95		3 043 3 478
								0 030	68	0 05	1 05	162	3 321	0 05	1 07		3 981 4 085
								0 080	80	0 05	82	143	1 430	0 11	1 09		2 213 2 208
109	1931-01-06																July 21 to August 20 ammonium chlorid, 129 grams Novasurol, 2 cc.
110	1931-01-06																Novasurol, 2 cc.
111	1931-01-06																January 12 to 20 ammonium chlorid 78 grams Novasurol, 1 cc.
112	1931-01-06																April 29 to May 13 ammonium chlorid, 150 grams Novasurol 1.5 cc.
113	1931-01-06	0 009	0 030	0 060	0 51			122	1 040	0 15	1 31	0 32	4 44	7 60		1 133	
114	1931-01-06	0 006	0 210	0 010	0 52			138	3 870	0 03	1 91	0 13	3 51	8 50		6 006	
115	1931-01-06	0 006	0 090	0 040	0 57			129	1 998	0 09	1 38	0 34	5 21	8 20		2 097	
116	1931-01-06							0 090	47	0 14	70	67	3 35	0 25	1 26		1 467 1 235
117	1931-01-06							0 010	52	0 02	85	130	4 810	0 04	1 63		6 075 5 975
118	1931-01-06							0 110	60	0 16	90	70	3 385	0 26	1 45		1 396 1 430
119	1931-01-06															August 13 to September 22 ammonium chlorid, 364 grams August 13 novasurol 2 cc.	
																Novasurol 2 cc.	
120	1931-01-06							0 040	33	0 14	1 08						September 28 to 30 sodium chlorid, 24 grams September 28 to October 1 increase in weight from 117.25 to 122.5 pounds
121	1931-01-06							0 010	39	0 02	1 18						
122	1931-01-06							0 090	58	0 11	0 67						
123	1931-01-06							0 050	36			39	413				
124	1931-01-06							0 060	36			68	408				
125	1931-01-06							0 030	38			102	510				
126	1931-01-06							0 060	25			115	460				
127	1931-01-06															March 13 to 21 ammonium chlorid 75 grams Novasurol 2 cc.	
128	1931-01-06																
129	1931-01-06	0 18	0 240	0 030	67			226	3 078								January 22 to 31 ammonium chlorid, 90 grams Novasurol 1 cc.
130	1931-01-06	0 07	0 210	0 009	0 25			228	6 473								
131	1931-01-06	0 03	0 140	0 030	83			163	2 615								
132	1931-01-06	0 001	0 010	0 050	15			70	3 091								February 25 to March 14 ammonium chlorid 146 grams March 18 to 22 ammonium chlorid, 48 grams and novasurol 1 cc.
133	1931-01-06	0 002	0 010	0 020	41			157	3 773								
134	1931-01-06	0 001	0 010	0 070	27			60	248								

<sup>1</sup> Calculated from the estimations of sodium, potassium, calcium, and magnesium.

TABLE 15  
The effect of hydrochloric acid on the blood and urine in a case of ascites and portal cirrhosis

Date, 1925	Sex	Age	C <sub>480</sub>	Blood		Urine		Salyrgan cc					
				Urea nitrogen mgm per cent	Chloride mgm per cent	Chloride mgm per cent	Volume cc	pH	S <sub>04</sub> grams as boracic sulphur as	Potassium grams	Ammonia nitrogen like FPN	Hydrochloric acid 10 per cent cc /10	Hydrochloric acid 10 per cent cc
September 1	M	36	8 0	64	338	700	7 2	2.77	0.182	0.336	1.89	1.23	1.337
September 2				1,200	7 0	3 02	0.180	0.411	2.26	1.51	1.514	1.192	1.186
September 3				900	7 2	2 160	0.783	0.369	1.71	1.17	1.207		1.360
September 4				700	7 0	2 020	0.728	0.413	1.94	1.02	1.225	0.210	1.299
September 5				800	6 8	2 300	0.850	0.440	2.35	1.10	1.520	0.330	1.375
September 6				750	6 4	3 300	0.810	0.450	1.85	1.24	1.630	0.580	1.326
September 7				600	7 2	2 920	0.630	0.390	1.60	1.13	1.020	1.210	1.504
September 8				750	5 8	5 040	0.810	0.113	0.89	2.20	1.088	1.310	1.48
September 9				6 4	44	370	4,850	5 622	0.580	0.776	10.04	5.87	6,063
										1.700	9.60	17.6	24.0

largest output of urine occurred in cases 22, 24 and 25 (table 14). In a period of between two and three weeks the actual volume of urine excreted daily by each of these patients on days of diuresis only, amounted to from 16.5 to 30 liters. In case 25 (table 14) the actual loss of weight during the five to six weeks of treatment was 70 pounds (31.8 kgm), and is mentioned to demonstrate the possibility of repeated marked diuresis in removing retained fluid from the body. On the other hand, two patients (cases 17 and 18, tables 10 and 13, fig. 6), showed no diuresis when the plasma chlorid level was below normal. The amount of mercury excreted in the urine by each of four patients on days of diuresis, when undergoing the combined treatment, was found to range from 48.5 to 81.3 per cent of that injected. The hydrogen-ion concentration of the urine showed no characteristic changes, though minor variations occurred. The chlorids excreted, except by patients with an abnormally low content of plasma chlorid, always showed a sustained or increased concentration, 0.36 to 0.70 gram per cent, and marked total increase. The maximal output of chlorid occurred in case 25 (table 14), 32.8 grams in twenty-four hours. Similarly, the sodium excreted showed an increased or sustained concentration, 0.05 to 0.44 gram per cent, and large total increase. The evenly sustained excretion of chlorid and sodium at a high level, during this twenty-four-hour period is of note. In case 25 when the amount of chlorid excreted in one period of twenty-four hours amounted to 32.8 grams the sodium excreted totalled 21.3 grams. Thus, on that particular day of the diuresis, total amounts of chlorid and sodium were excreted in the proportions of 6.4, the ratio of the two ions in the salt, sodium chlorid. We shall point out that such a ratio of the ions does not always occur. Potassium is usually excreted at a lower concentration during days of diuresis, but the total output is sustained or increased. The variations in the excretion of potassium, particularly in relation to the excretion of chlorid and sodium, are well exemplified during two periods of diuresis, August 16 and 18, in case 25 (table 14). In the two experiments the total volume of urine was the same and the total chlorid differed by only 2 grams, whereas the sodium differed by 10 grams and the potassium by 3.7 grams. Thus when the chlorid excreted was stationary, there was a fall of 10 grams in the sodium

but an increase of 37 grams in the potassium. Whereas in the first experiment almost all the chlorid was excreted as sodium chlorid, in the second the total excretion of sodium and potassium combined did not furnish sufficient base for the large output of chlorid. The

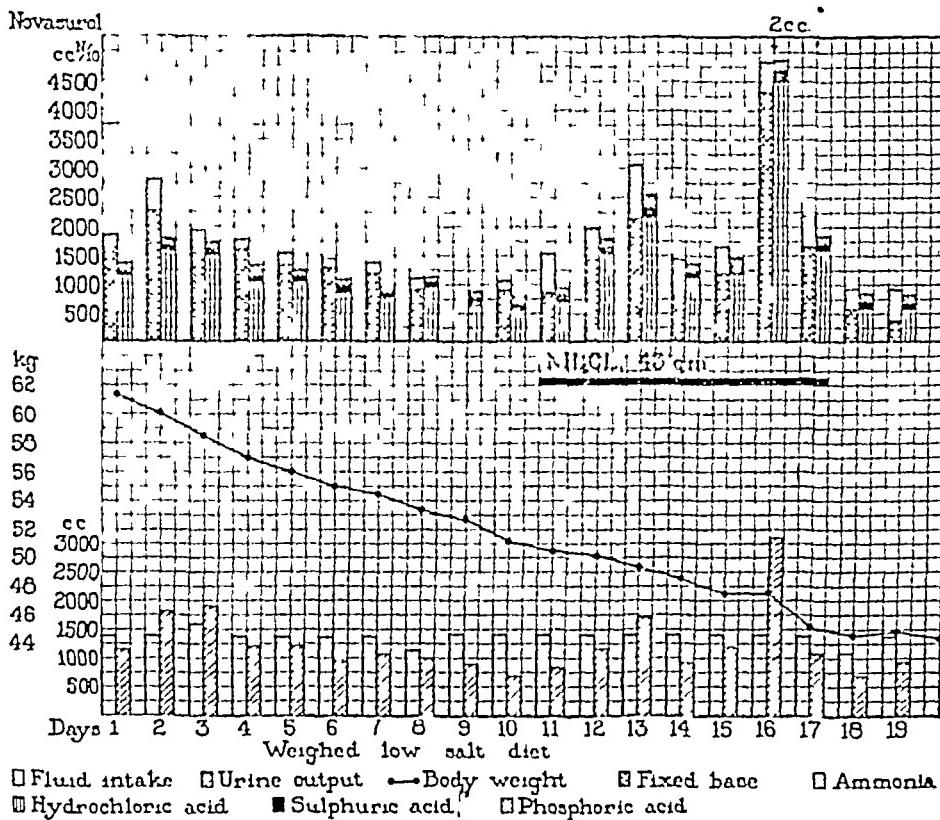


FIG 3 CHRONIC NEPHROSIS RELATIVE DIURETIC EFFECT OF AMMONIUM CHLORID ALONE AND AMMONIUM CHLORID PLUS NOVASUROL, ALSO THE SUM OF THE INORGANIC ACID AND INORGANIC BASIC IONS PLUS AMMONIA IN THE URINE CALCULATED AS ONE-TENTH NORMAL CUBIC CENTIMETERS, ACID OR ALKALI (CASE 4, TABLES 3, 4, 13 AND 14)

ammonium radical unfortunately was not determined. The ammonia excreted was always less concentrated on the days of diuresis, whether the total amount was slightly decreased, maintained the same, or slightly increased. The total fixed base was always markedly increased and the concentration maintained or raised, thus paral-

leling the chlorid excreted. The total calcium excreted was invariably increased and the magnesium also usually. The sulphate output showed a fall in concentration with a small increase in total amount. A fall in concentration with the total output unchanged was characteristic of the phosphate excretion, a result similar to that noted with

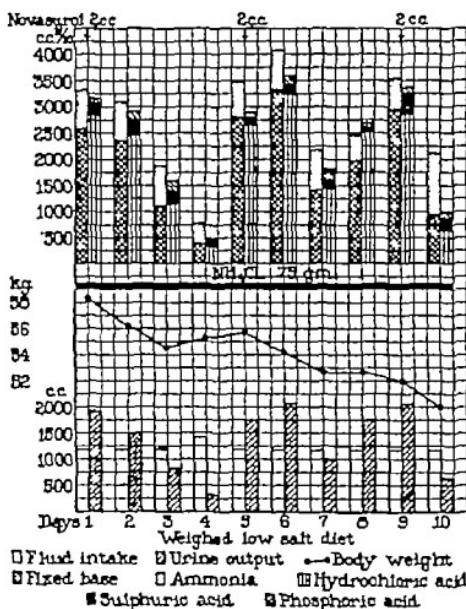


FIG. 4 CHRONIC ENDOCARDITIS AND POLYCYTHEMIA PROLONGED, COMBINED DIURETIC EFFECT OF AMMONIUM CHLORIDE AND NOVASUROL, ALSO THE SUM OF THE INORGANIC ACID AND INORGANIC BASIC IONS PLUS AMMONIA IN THE URINE CALCULATED AS ONE TENTH NORMAL CUBIC CENTIMETERS, ACID OR ALKALI (CASE 7, TABLES 13 AND 14)

novasurol alone. Similarly also there were no constant changes observed in the output of urea or total nitrogen.

Comparison of the excretion of the total inorganic acid ions, with the inorganic basic ions and ammonia, expressed as one tenth normal acid or alkali, in a normal subject (case 8, table 14, fig 2) shows a preponderance of the inorganic acid ions. A similar finding, but with increase of acid over base less marked, was noted in case 4 (table 14,

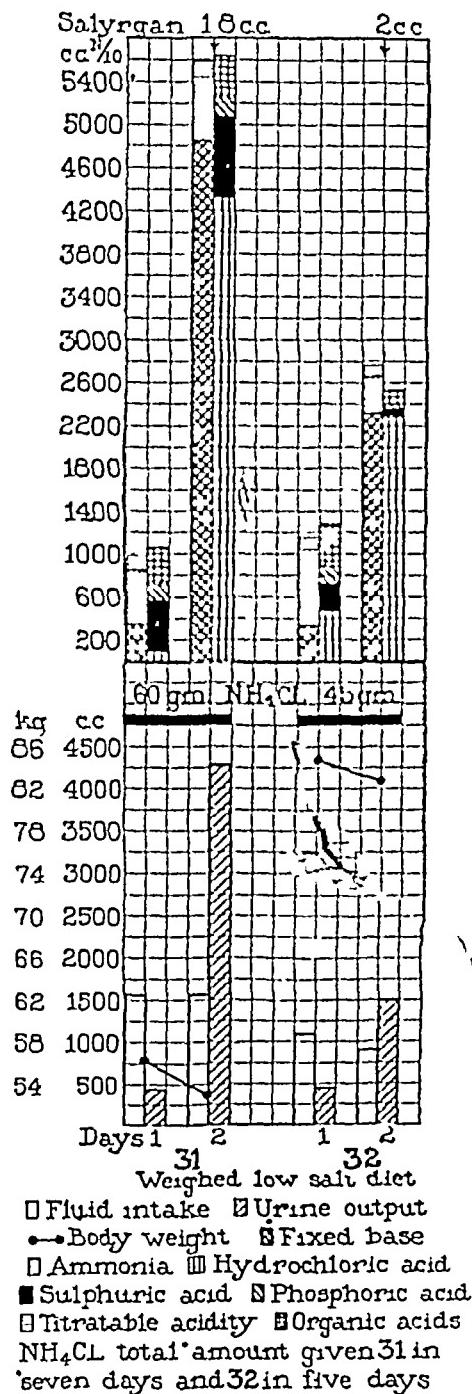


FIG 5 RELATION OF THE SUM OF ACID AND BASIC IONS IN THE URINE CALCULATED AS ONE-TENTH NORMAL CUBIC CENTIMETERS, ACID AND ALKALI (CASES 31 AND 32, TABLE 16)

TABLE 16  
The effect of novastrol and ammonium chloride on the acid-base factors in urine

Case	Age	Sex	Diagnosis	Date	Chlorid	Isotonic molarity as $\text{SO}_4$	Phosphorus	Organic acids	Titratable acidity	Ammonium nitrogen	Total bases $\text{m}/10 \text{ cc.}$													
											C $\text{C} \text{m}/10$	C $\text{C} \text{m}/10$												
31	42	F	Portal cirrhosis	October 30	700	5.4	0.070	0.50	0.279	95	0.055	0.345	48.7	139.2	24.0	168.0	0.090	693	47.0	322	1.013	81.7		
				October 31 <sup>a</sup>	4250	4.8	0.316	15.56	0.069	96	0.009	0.383	9.2	391.0	3.5	150.0	0.019	808	112.8	1.866	5.600	5.443		
32	49	F	Cystadenoma of ovary	November 23	400	5.0	0.426	1.704	0.271	1.03	0.091	0.364	106.0	424.0	33.2	13.5	0	238.0	952	91.0	364	1.237	1.044	
				November 24 <sup>b</sup>	500	4.6	0.546	8.19	0.018	27	0.003	0.048	9.9	1113.0	7.0	103.0	0.079	0.0	435	156.0	0.2	310	2.503	1.631

Phosphorus calculated as 0.1 mgm. in total acids.

<sup>a</sup> Salogen 1.5 cc.

<sup>b</sup> Salogen 2 cc. Ammonium chloride, 9 grams daily. Case 31 October 26 to November 6 case 32 November 18 to December 3

fig 3) In case 7 (fig 4) and case 24 (table 14), the total inorganic acid closely approximated the total basic ions. The phosphate ion was calculated as one-tenth molar solution, it thereby having been assumed that it had bound one equivalent of base.

Two patients with marked ascites (case 31 and 32) with only an occasional trace of albumin in the urine manifested definite diuresis after the combined treatment. The titratable acid, and total organic acids were determined in addition to the total inorganic acid, basic ions and ammonia (table 16 and fig 5). The amount of the total acid excreted closely parallels that of the total base and the titratable acid. These results again emphasize the importance of the specific excretion of chlorid along with fixed base when diuresis is produced by the combined action of ammonium chlorid and organic mercury compounds.

#### OTHER DIURETICS

The urinary findings of two patients with diuresis after the use of theocin, digitalis, and novasurol are recorded in table 17. In the diuresis produced by theocin and digitalis there is an increase in chlorid, sodium and fixed base similar to that produced by novasurol. Steyrer reported diuresis with increase in the chlorid excreted following the administration of digitalis to a patient with cardiac compensation. Katsuyama and Pototski noted that after caffeine derivatives had been administered to rabbits, the chlorid and sodium excreted in the urine were increased. Bock found that, besides the increase in the chlorid and sodium, the potassium content of the urine was increased during caffeine diuresis. Meinertz first reported an increase in the chlorid excreted after the use of caffeine derivatives in clinical cases of edema. In the single case in this series in which all three diuretic agents were administered (case 14, table 17), novasurol brought about the maximal excretion of chlorid, sodium and fixed base, although the amount of water excreted equaled that after digitalis. Jendrassik, in 1891,<sup>6</sup> gave calomel to a series of patients

<sup>6</sup> Jendrassik's advocacy of the use of calomel for its diuretic effect was based on certain remarkable instances of diuresis obtained in cases of obstinate edema in Wagener's clinic in Budapest. The calomel treatment for edema was given a trial in numerous clinics at this period, but because of the coexisting diarrhea, and at times harmful effects on the kidney, its use as a diuretic was generally abandoned.

TABLE 17  
*A comparison of the diuretic effect of digitalis, theocin and novasurol*

S J	E G	M	Diagnosis	Date 1933	Volume, ml.	pH	Chlorid			Sodium			Phosphate			Total urine basic base		Dosage
							Grams per cent	Total, grams	Grams per cent	Grams per cent	Total, grams	Grams per cent	Grams per cent	Total, cc./10	Cc. x 10 per cent	Total, grams	Grams per cent	Theocin, 0.7 gram
14	51	M	Myocardial degeneration, decompensation and portal cirrhosis	July 6	500	7.00	55	2.74	31.1	56.0	110.0	57	202	1.012	Theocin, 1.0 gram			
				July 7	800	7.00	53	4.34	44.3	56.0	050.0	41	242	1.936	Theocin, 1.0 gram			
				July 8	1,400	6.80	23	3.30	0.182	54.0	020.0	28	124	1.730	Tincture of digitalis, 8 cc.			
				July 9	3,000	5.80	31	9	18.0	11.3	27.0	015.0	45	122	3.675	Tincture of digitalis, 8 cc.		
				July 10	1,150	6.00	52	5.94	0.141	59.0	030.0	37	190	2.185	Tincture of digitalis, 8 cc.			
				July 11	1,250	5.60	46	5.70	28.3	50.0	044.0	55	200	2.500	Tincture of digitalis, 8 cc.			
				July 12	2,800	5.20	55	15.50	0.308	29.0	014.0	39	178	4.984	Novasurol, 2 cc.			
				July 13	700	5.40	46	3.19	0.221	55.0	040.0	26	182	1.374				
				July 14														
33	64	M.	Myocardial degeneration, decompensation and auricular fibrillation	June 22	4,400*													
				June 23	1,700†											205	3.485	

\* Diet, 400 cc. milk.  
 † Diet, 800 cc. milk.

with cardiac edema and noted a similar increase in the urinary excretion of water and chlorids

*Comparative findings in the blood and urine* In three patients with marked edema (cases 7, 22 and 24, table 14) who were given the combined treatment a great diuresis occurred when the carbon dioxide combining-power of the plasma was very slightly below the normal, 48 per cent by volume, and the blood urea was within normal limits, but the plasma-chlorid level was definitely increased Urinalysis revealed (1) acidity of the urine with little if any change in hydrogen-ion concentration from the previous day, (2) marked increase in chlorid, sodium and total fixed base, (3) a small but definite increase in ammonia, (4) very close balance between the total inorganic acid ions, and those of the total inorganic base with ammonia included, and (5) in one case (case 22) an increase in the urea and total nitrogen The only distinct difference between these results and those of the normal subject (case 8, table 14) is the absence in the former cases of a distinct decrease in the alkali reserve of the plasma

Cases 17 and 18 (tables 10 and 13) clearly show the close relationship between a low level of plasma chlorid and the consequent low urinary excretion of chlorid and the diuretic action of novasurol With the level in the plasma below normal and in the presence of marked edema, practically no excretion of chlorid occurred nor did diuresis take place, but when the content was raised to the normal level in case 18 by administering ammonium chlorid, chlorid, fixed base and water were excreted in large amounts There was no definite relationship between decreased alkali reserve in the plasma and active diuresis, the latter occurring when the former varied between 37.5 and 76 per cent by volume (fig. 6)

The effect of sodium chlorid, as contrasted with that of ammonium chlorid, in an organism which tends to retain fluid readily, is well shown in case 25 (table 14) The patient's weight had been reduced from 84 to 53 kgm (185 to 117 pounds) by the combined use of ammonium chlorid and novasurol For three days he was given a total of 24 grams of sodium chlorid, but the volume of the urine and the chlorid, sodium, and fixed base excreted remained approximately stationary, nor did the low plasma chlorid show any change The patient gained 2.4 kgm in weight These results clearly indicate

that sodium chlorid in this case passed readily to and lodged in the tissues generally, carrying with it a certain amount of water, hence the increase in weight. This clinical experiment closely parallels that reported by Widal and Javal in a case of edema with chronic nephrosis.

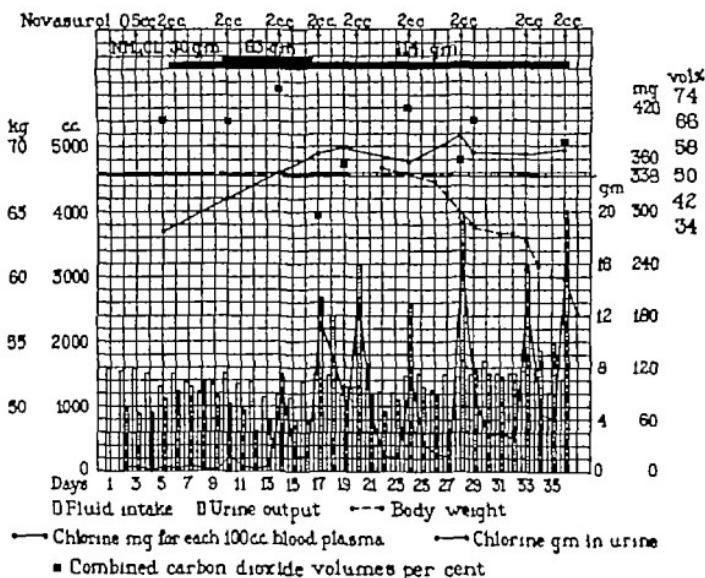


FIG 6 RELATIONSHIP OF AN ABNORMALLY LOW AND NORMAL PLASMA CHLORID TO THE DIUREYSIS PRODUCED BY AMMONIUM CHLORID AND NOVASUROL.  
(CASE 18 TABLES 10, 13 AND 14)

## DISCUSSION

*Action of ammonium chloride* Following the injection or absorption of this salt the ammonium ions are rapidly synthesized into urea with consequent increase in the blood urea and the urea excretion. The ammonium ions are thus rapidly removed from the blood and tissues leaving hydrochloric acid which causes a shift of the buffer systems toward the acid side. Haldane and Gamble have stressed the chlorid acidosis as the important factor in the causation of the diuresis. Besides the acidosis, we have been led to consider the increase of urea

and the increase per se of chlorid in the blood and tissues, as possible factors in inducing diuresis

If the resulting acidosis is the sole cause of the diuretic action of ammonium chlorid the ingestion of acids should have a similar effect Begun, Herrmann and Münzer studied the effect of the ingestion of a 12.3 per cent solution of hydrochloric acid, 170 and 85 cc, respectively, in two normal men. During the period of acid ingestion they observed an increase in the excretion of chlorid, ammonia nitrogen and titratable acid in the urine. In one case there was no diuresis, and in the other the moderate increase in the volume of urine was due to an increase in the intake of water. One of our patients (case 30, table 15) was given, by mouth, 116 cc of 10 per cent hydrochloric acid, in daily amounts varying from 8 to 48 cc for four days. This amount of hydrochloric acid contains 11.3 grams chlorin, equal to that in 16.5 grams of ammonium chlorid. The ingestion of the acid caused a very slight increase in the plasma chlorid and decrease in carbon dioxide combining-power of the plasma. Studies of the urine showed that the acid had no definite diuretic action. The excretion of chlorid and ammonia was increased. Sodium and potassium showed some variation for it seemed that an increase in sodium was accompanied by a decrease in potassium and vice versa, while the total fixed base excretion remained constant. In this experiment the acid ingestion produced evidences of acidosis without diuresis, however, the lack of increase in the volume of the urine may have been due to the relatively small amount of acid ingested. These results with hydrochloric acid and the fact that the normal subject (case 8) developed acidosis without diuresis in three days after taking 31.5 grams of ammonium chlorid, indicate clearly that acidosis can be produced by chlorid without a parallel increase in the volume of urine. Further experiments with other inorganic acids as sulphuric and nitric acids, are needed to decide the question as to whether such disturbance in the acid-base equilibrium towards the acid side in the tissues will cause liberation of water. In interpreting such experiments the possible specific action of the anion must also be considered.

An abnormally low level of plasma chlorid with little or no chlorid excreted in the urine has been demonstrated in cases of severe renal

insufficiency with and without demonstrable edema, lobar pneumonia, extensive superficial burns, war gas poisoning, the toxic syndrome associated with upper intestinal obstruction, and in two cases of portal cirrhosis with edema and ascites in this series (cases 17 and 18, table 10). In one of the latter (case 18) when the ascites and edema were extensive the ingestion of ammonium chlorid gradually raised the lowered chlorid plasma threshold to the normal level and thus permitted an increase in excretion through the kidney.

At first glance the administration of a chlorid in large amounts to an organism literally loaded down with chlorids and water seems most illogical. However, it proved to be a life-saving measure in the case just described. The exact manner in which the chlorid current was thus diverted from the general tissues toward the blood stream and hence through the kidney is not easily explained. Sodium chlorid had no such effect in case 25 (table 14). On the other hand, it augmented the current of chlorid and water toward the tissues. This simple clinical experiment shows the important relationship between retained sodium and retained water, as pointed out by Meyer and Blum. Besides the acidity factor, due to ammonium chlorid, the status quo of the ions already present, and the specific action of the chlorid ion itself must also play a part in causing such an important shift in the movement of the body fluids.

*Action of organic mercury compounds.* A large percentage of the injected mercury is excreted in the urine. Diuresis is accompanied by an increased excretion of chlorid and fixed base. The latter is chiefly due to the increased elimination of sodium although potassium in certain instances made up a considerable fraction. The urine excreted the day following diuresis contained small amounts of chlorid and sodium, but the average amount of potassium. The latter urinary findings are similar to those noted by Benedict during starvation, in which condition there is known to be a retention of chlorid and sodium. There was no conclusive evidence during this diuresis of any marked change in the acid base equilibrium of the blood or urine. We did note the absence of diuresis when the plasma chlorid content was well below the normal threshold. Further we have been unable to show any constant changes in the composition of the blood during the period when a large quantity of fluid must

necessarily be liberated from the tissues and transported within the organism. These negative findings are in harmony with those of Haldane and Priestley in the blood of normal man after the ingestion of a large quantity of water. Greene and Rountree had to give a much larger amount of water to animals to produce changes in blood concentration. The liberation of ascitic fluid by organic mercury compounds in cases of serious hepatic disease without any demonstrable renal impairment would lead one to believe that the specific action was in the tissues remote from the kidneys. The specific action in renal edema suggests an effect both on the general tissues and kidneys. Experiments on the perfused isolated kidney might be crucial in determining the specific site of action.

*Combined action of ammonium chlorid and organic mercury compounds.* Following the combined administration of ammonium chlorid and organic mercury compounds, marked diuresis was the rule. The diuresis occurred more consistently than before, when the diuretic substances were given singly, and large accumulations of fluid within the body were removed in a comparatively short period. Both substances have the same fundamental property of removing chlorid and fixed base as well as water from the organism, and their combined action may thus be simply a cumulative one. Full diuresis did occur when the blood plasma values for urea, chlorid and the carbon dioxide combining-power were normal. However, even with the normal plasma content of urea, chlorid and carbon dioxide combining-power, the urinary findings showed an increased excretion of ammonia which indicated definite acidosis. Such results point to a possible compensatory regulation of the acid-base equilibrium in the plasma when the tissues are still more acid in reaction than normal. Thus the acidosis produced by an acid-forming salt, after a long period of ingestion, may be closely analogous to a case of compensated acidosis in diabetes mellitus. In our experience novasurol alone did not cause a demonstrable change in the acid-base equilibrium of the body, whereas when combined with ammonium chlorid there was always evidence, in the plasma or urine, of acidosis. We have thus demonstrated that ammonium chlorid can produce acidosis in the organism with or without diuresis, that novasurol can bring about diuresis when there is no acidosis, and that the combined

exhibition of ammonium chlorid and novasurol causes the most marked diuresis when acidosis is present. From such facts one must conclude that an abnormal acid reaction in the tissues need not per se cause diuresis, but since it occurs when the diuretic response is most regular and marked, it must be considered a possible factor in liberating water from the tissues.

When diuresis follows the use of the caffeine diuretics and digitalis the urine shows a decided increase in the chlorid and fixed base excreted. If acidosis favors diuresis, the combined use of an acid-forming salt and theocin, for example, might cause diuresis after theocin alone has failed. We are at present carrying out some experiments to test this possibility.

Urea has long been recognized as possessing diuretic properties and has been used as a therapeutic agent in different types of edema. Crawford and McIntosh have lately reported beneficial effects from its use in cases of cardiac edema. Their results show clearly that the increased elimination of water is accompanied by an increased excretion of urea, but with only slight irregular increases in chlorids. Here then the mechanism of diuresis is quite distinct from that involved when ammonium chlorid or an organic mercury compound, caffeine or digitalis, is administered. Similarly, hypertonic solutions of glucose and saccharose have been employed experimentally to produce marked diuresis and subsequent dehydration. Some of our unpublished data indicate that, like urea, these sugars may not necessarily cause a distinctly increased excretion of chlorid and fixed base. From a practical therapeutic standpoint those substances which remove water, chlorid, and fixed base from edematous patients remove both the retained fluid and its inorganic dissolved constituents and therefore accomplish a more complete withdrawal than diuretics of the urea and sugar type.

The removal of accumulated fluid, in cases of cardiac and nephritic edema, and in cases of ascites associated with disease of the liver, is a fundamental problem in therapeutics. This excess fluid is either retained within the cells themselves, in the tissues or in the body cavities. It may leave the cells, tissues or body cavities spontaneously. Digitalis, the caffeine derivatives, acid forming salts, organic mercury compounds or urea may bring about diuresis when it does not

occur spontaneously. The site of action of these diuretics would seem to be diffuse and not limited to one specific organ, the kidney. The exact quantitative levels at which salts can pass from the edematous tissues into the blood stream and from the blood stream through the kidney may be affected by these drugs. That abnormal levels do exist in edematous conditions and can be altered has been well shown in cases of this series. These diuretics may also act by permitting salts and water to pass through cells previously impervious, as the renal cell in oliguria and the peritoneal endothelium in the ascites of cirrhosis of the liver.

#### SUMMARY

Following the administration of ammonium chlorid and its absorption into the blood stream it has been shown experimentally that the ammonia is quickly synthesized to urea. The liberated hydrochloric acid increases the chlorid content of the blood and tissues with the production of acidosis. Under the conditions of these experiments diuresis may or may not occur after the exhibition of an adequate amount of ammonium chloride. Ammonium chlorid in man causes an increased excretion of chlorid, fixed inorganic base (particularly sodium and potassium), ammonia, urea, and total nitrogen in the urine.

Organic mercury compounds cause an increased excretion of chlorid and inorganic fixed base, without evidence in the blood or urine of a change in acid-base equilibrium. The basic ions, sodium and potassium, may be excreted independently. Diuresis occurs usually but not always. Changes in the concentration of the blood are inconstant and never marked in degree, even when the diuresis is great. Eighty-five per cent of the injected mercury can be recovered in the subsequent twenty-four-hour specimen of urine.

Ammonium chlorid and organic mercury compounds used in combination produce diuresis when singly they fail to do so. They cause acidosis, an increase in the chlorid content of the blood, and an increase in the excretion of water, chlorid, fixed inorganic base, chiefly sodium and potassium, and ammonia. In one case in which there was a subnormal plasma chlorid level the combined drugs failed to

produce diuresis. Ammonium chloride was then given until the chlorides reached the normal level, when organic mercury produced satisfactory diuresis.

The evidence at hand indicates that the activity of these diuretics is not limited to the kidney, but that extrarenal factors enter into consideration.

#### BIBLIOGRAPHY

- 1 Baird, N. M., Douglas, C. G., Haldane, J. B. S., and Priestley, J. G. *Jour Physiol.*, 1922-1923, lvi, xli. Ammonium Chloride Acidosis
- 2 Begun, A., Herrmann, R., Münzer, E. *Biochem Ztschr.*, 1915, lxxi, 255-267 Über Acidosis und deren Regulation im menschlichen Körper
- 3 Bell, R. D., and Doisy, E. A. *Jour Biol. Chem.*, 1920, xliv, 55-67 Rapid Colorimetric Methods for the Determination of Phosphorus in Urine and Blood.
- 4 Benedict, F. G. Washington, D. C., Carnegie Institute, 1915, No. 203, p 285 A Study of Prolonged Fasting
- 5 Bleyer, Leo. *Klin Wchnschr.*, 1922, 1, 1940-1942 Erfahrungen über die Novasurol-diurese.
- 6 Blum, L. *Verhandl. d. Kong f. inn Med.*, 1909, 122-126 Ueber die Rolle von Salzen bei der Entstehung von Oedemen
- 7 Bock, J. *Skandinav Arch f. Phys.*, 1911, xxv, 239-246 Über die Ausscheidung der Alkalimetalle bei der Purindiurese.
- 8 Bohn, Hans. *Klin Wchnschr.*, 1923, li, no. 1, 352-353 Experimentelle Studien über die diuretische Wirkung des Novasurols.
- 9 Bollman, J. L., Mann, F. C., and Magath, T. B. *Amer Jour Physiol.*, 1924, lxix, 371-392 Studies on the Physiology of the Liver VIII. Effect of Total Removal of the Liver on the Formation of Urea
- 10 Boothby, W. M., and Sandiford, Irene Philadelphia, Saunders, 1920, 117 pp Basal Metabolic Rate Determinations
- 11 Briggs, A. P. *Jour Biol. Chem.*, 1922, lii, 13-16 A Modification of the Bell Doisy Phosphate Method.
- 12 Crawford, J. H., and McIntosh, J. F. *Arch. Int. Med.*, 1925, xxxvi, 530-541 The Use of Urea as a Diuretic in Advanced Heart Failure
- 13 Crawford, J. H., and McIntosh, J. F. *Jour Clin. Invest.*, 1925, I, 333-358 Observations on the Use of Novasurol in Edema Due to Heart Failure.
- 14 Fiske, C. H. *Jour Biol. Chem.*, 1922, li, 55-61 A Method for the Estimation of Total Base in Urine
- 15 Folin, Otto. *Jour Biol. Chem.*, 1905, i, 150-159 The Determination of Sulphates and Total Sulphur in Urine.
- 16 Folin, Otto, and Bell, R. D. *Jour Biol. Chem.*, 1917, xxix, 329-335 Applications of a New Reagent for the Separation of Ammonia I. The Colorimetric Determination of Ammonia in Urine

- 17 Folin, Otto, and Wu, Hsien Jour Biol Chem, 1919, xxviii, 81-110 A System of Blood Analysis
- 18 Grable, J L, Blackfan, K D, and Hamilton, Bengt Jour Clin Invest, 1925, i, 359 A Study of the Diuretic Action of Acid Producing Salts
- 19 Gillespie, L J Amer Chem Soc Jour, 1920, xlii, 742-748 Colorimetric Determination of Titration Curves Without Buffer Mixtures
- 20 Greene, C H, and Rountree, L G Amer Jour Physiol, 1924, lxviii, 111 Changes in the Concentration of the Blood Following the Administration of Excessive Quantities of Water
- 21 Haldane, R L Jour Lab and Clin Med, 1923, viii, 411-414 A Method for the Determination of Hemoglobin
- 22 Haldane, J B S Jour Physiol, 1921, iv, 265-275 Experiments on the Regulation of the Blood's Alkalinity
- 23 Haldane, J B S, and Priestley, J G Jour Physiol, 1915-1916, i, 296-303 The Regulation of Excretion of Water by the Kidneys I
- 24 Harvey, S C Arch Int Med, 1910, vi, 12-18 The Quantitative Determination of the Chlorids in the Urine
- 25 Jendrassik, E Deutsch Arch f klin Med, 1885-1886, xxviii, 499-524 Das Calomel als Diureticum
- 26 Jendrassik, E Deutsch Arch f klin Med, 1890-1891, xlvii, 226-288 Weitere Untersuchungen über die Quecksilberdiurese
- 27 Katsuyama, K Ztschr f physiol Chem, 1899, xxviii, 587-594 Ueber der Einfluss des Theins auf die Ausscheidung von Alkalien im Harne
- 28 Katsuyama, K Ztschr f physiol Chem, 1901, xxxii, 235-240 Ueber den Einfluss einiger harntreibender Mittel auf die Ausscheidung von Alkalien im Harne
- 29 Keith, N M Amer Jour Physiol, 1924, lxviii, 80-96 Experimental Dehydration Changes in Blood Composition and Body Temperature
- 30 Keith, N M, Barrier, C W, and Whelan, Mary Jour Amer Med Assn, 1925, lxxxv, 799-806 The Diuretic Action of Ammonium Chlorid and Novasurol in Cases of Nephritis with Edema
- 31 Keith, N M, Smith, Florence, and Whelan, Mary Arch Int Med, 1926, xxvii, 550-558 The Therapeutic Use of Diets Low in Water and Mineral Content
- 32 Keith, N M, and Thomson, W W D Quart Jour Med, 1918, xi, 229-266 War Nephritis A Clinical, Functional and Pathological Study
- 33 Keith, N M, and Whelan, Mary Amer Jour Physiol, 1925, lxxii, 195-196 The Effect of Novasurol on the Composition of Blood and Urine
- 34 Kramer, Benjamin, and Tisdall, F F Jour Biol Chem, 1921, xlvi, 339-349 A Clinical Method for the Quantitative Determination of Potassium in Small Amounts of Serum
- 35 Kramer, Benjamin, and Tisdall, F F Jour Biol Chem, 1921, xlvi, 467-473 A Simple Method for the Direct Quantitative Determination of Sodium in Small Amounts of Serum

- 36 Kramer, Benjamin, and Tisdall, F F *Jour Biol. Chem.*, 1921, **xlvii**, 475-481 A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum
- 37 Lewis, D S, and Rivers, T M *Bull Johns Hopkins Hosp.*, 1916, **xxvii**, 193-201 Chemical Studies on a Case of Bichloride Poisoning
- 38 MacCallum, W G Lintz, Joseph, Vermilye, H N, Leggett, T H, and Boas, E *Bull Johns Hopkins Hosp.*, 1920, **xxx**, 1-7 The Effect of Pyloric Obstruction in Relation to Gastric Tetany
- 39 Meinertz, J *Therap Monatsh.*, 1904, **xviii**, 275-285 Versuche über Diurese insbesondere über die Wirkung des Theocinnatrium aceticum
- 40 Meyer, L F *Verhandl. d. Versamml. d. Gesellsch f. Kinderh.*, 1909, **xiv**
- 41 Moraczewski, v W *Arch f path Anat. u Physiol u f Klin Med.*, 1896, **cxvi** 424-452 Ueber den Chlor und Phosphorgehalt des Blutes bei krankhaften Zuständen
- 42 Mühlung, Adolf *München med Wchnschr.*, 1921, **lxviii**, no 2, 1447-1449 Studie über die diuretische Wirkungsweise von Quicksilber Ausgeführt mit dem organischen Quecksilberpräparat Novasurol
- 43 Nash, T P, and Benedict, S R *Jour Biol. Chem.*, 1921, **xlviii**, 463-488 The Ammonia Content of the Blood and Its Bearing on the Mechanism of Acid Neutralization in the Animal Organism
- 44 Nonnenbruch W *München med Wchnschr.*, 1921, **lxviii**, n, 1282-1283 Ueber die Wirkung des Novasurols auf Blut und Diurese.
- 45 Potozky, C *Arch f d ges. Physiol.*, 1902, **xci**, 584-594 Beiträge zur Diurese. Ueber den Einfluss einiger Diuretica auf die Kochsalzausscheidung, insbesondere beim Kochsalzarmen Thiere
- 46 Priestley, J G *Jour Physiol.*, 1915-1916, **1**, 304-311 The Regulation of Excretion of Water by the Kidneys. II.
- 47 Rowntree L G, Keith, N M, and Barrier, C W *Jour Amer. Med. Assn.*, 1925 **Lxxv**, 1187-1193 Novasurol in the Treatment of Ascites in Hepatic Disease.
- 48 Saxl, P, and Heilig R *Wien Klin Wchnschr.*, 1920, **xxxiii**, 943 Ueber die diuretische Wirkung von Novasurol und anderen Quecksilberinjektionen
- 49 Smith, Millard *Jour Biol. Chem.*, 1921, **xlv**, 437-447 The Determination of Chlorides in Trichloro-acetic Acid Filtrates from Whole Blood and Plasma.
- 50 Steyrer *Beitr z. chem Physiol. u Path.*, 1902, **u**, 312 Über osmotische Analyse des Harns.
- 51 Tisdall, F F, and Kramer, Benjamin *Jour Biol. Chem.*, 1921, **xlviii**, 1-12 Methods for the Direct Quantitative Determination of Sodium, Potassium, Calcium and Magnesium in Urine and Stools.
- 52 Underhill, F P *The Lethal War Gases*. New Haven, Yale University Press, 1920

- 53 Underhill, T P , Carrington, G L , Kropsinow, Robert, and Pack, G T  
Arch Int Med , 1923, xxxii, 31-49 Blood Concentration Changes in  
Extensive Superficial Burns, and Their Significance for Systemic Treatment
- 54 Van Slyke, D D , and Cullen, G E Jour Biol Chem , 1914, xix, 211-228  
A Permanent Preparation of Urease, and Its Use in the Determination  
of Urea
- 55 Van Slyke, D D , and Palmer, W W Jour Biol Chem , 1920, xl, 567-585  
Studies on Acidosis XVI The Titration of Organic Acids in Urine
- 56 Van Slyke, D D , and Stadie, W C Jour Biol Chem , 1921, xlii, 1-42  
The Determination of the Gases of the Blood
- 57 Whelton, Mary Jour Biol Chem , 1925, lxvi, 585-620 The Effect of  
Intravenous Injection of Inorganic Chlorides on the Composition of Blood  
and Urine
- 58 Widal, F , and Javal, A Bull et mém Soc de med d hôp de Par , 1903,  
3 s , xx 733-749 La cure de dichloururition, son action sur l'œdème,  
sur l'hydratation et sur l'albuminurie à certaines périodes de la néphrite  
épithéliale
- 59 Wong, San Yin Jour Biol Chem , 1923 lv, 431-435 The Use of Persulfate  
in the Estimation of Nitrogen by Folin's Direct Nesslerization Method

## BENIGN GLYCOSURIA DUE TO DISTURBANCES IN THE BLOOD SUGAR REGULATING MECHANISM<sup>1</sup>

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Chr Bohr, the Danish physiologist, always emphasized the importance of making an exact study of the adjustments exhibited by the organism to adapt the functions of the various organs to each other, and thereby to ensure the equilibrium of the organism necessary for maintaining a healthy, undisturbed life.

Among these adjustments that of the blood sugar is one of the most interesting, both on account of its great significance in ensuring an undisturbed metabolism and also because quite minor disturbances in its regulation manifest themselves by abnormal phenomena in the individual.

Besides the liver, acting as a reservoir between the intestine and the blood, one of the best known means possessed by the organism for the maintenance of a certain concentration of glucose in the blood is the sugar threshold. Since Claude Bernard's experiment in the middle of the last century, it has been known that glucose is not excreted in the urine until it exceeds a certain concentration. Ambard and after him Cushny thoroughly examined the "problem of threshold" in detail. After Bang had introduced the micro-method for the determination of blood sugar, Jacobsen (15) in our clinic found the value of the threshold in normal persons to be about 0.16 to 0.18 per cent of blood sugar. A number of investigators have subsequently confirmed this, in so far as they have demonstrated that this is the usual threshold value in normal individuals but on the other hand, higher or lower values are by no means rarely observed. Subsequently it was proved that the blood sugar level is not

<sup>1</sup> Herter Lecture given in Baltimore, February 18, 1926

constant. On the contrary the blood sugar rises after every meal containing carbohydrates and follows in the course of the

TABLE I  
*Normal female, 21 years, weight 60 kgm Blood sugar, mgm per 100 cc*

Time	Ear	Vein
9 05	85	85
15	103	
25		124
25	156	
45		111
55	119	
10 05		91
15	119	
30	80	
35	85	
45		67
55	93	
11 05		65
15	76	
28		58
35	64	

At 9 08, 60 grams of glucose in 600 cc. of water

TABLE 2  
*Diabetic female, 65 years, weight, 98 kgm Blood sugar, mgm per 100 cc*

Time	Lar	Vein
11 30	128	128
50	193	
12 00	212	
10	237	
17	237	
20	236	
22	235	
30	229	
40	225	
1 30	173	

At 11 30, 42 grams glucose in 250 grams water

day a curve with several peaks, which are dependent upon the time and number of meals. Jacobsen (14) was the first to demonstrate that in human beings this variation occurs not only

after the ingestion of glucose but also after ordinary meals containing carbohydrates. The greater the quantity of carbohydrates ingested the higher the blood sugar rises, both after the ingestion of sugar and of starch. When the alimentary hyperglycemia rises above the threshold for blood sugar, glycosuria sets in. The rise in the alimentary hyperglycemia, however, is not proportional to the quantity of the carbohydrates ingested.

Important investigations in this field have been conducted in Denmark by Hagedorn (7) and Karen Marie Hansen. Hagedorn (8) has demonstrated that when a person is fasting the percentage of blood sugar is equally high in arterial and venous blood, but after ingestion of carbohydrates the rise in blood sugar is considerably higher in arterial than in venous blood. During the circulation through the capillaries a removal of the blood sugar in the peripheral tissues takes place. This is not seen in true diabetes where there is no difference, or much less, between the blood sugar in the arteries and the veins. Hansen (10) has furthermore shown that the organism possesses the capacity to accelerate the removal of blood sugar when the blood sugar otherwise would rise abnormally high. As a result of this regulating capacity the blood sugar in normal individuals never exceeds a certain maximum which Hansen terms the optimum concentration. In normal individuals this does not, as a rule, exceed 0.18 per cent. The blood sugar after ingestion, for instance, of 50 grams of glucose, rises to 0.18 per cent and it does not rise any higher after ingestion of 100 grams, or even after ingestion of 200 or 400 grams of sugar. This is beautifully illustrated by the four curves shown in figure 1. They are all taken from the same individual. In five normal individuals Hansen found the highest value to which the blood sugar rose after the ingestion of 200 to 400 grams of glucose to be 0.16 to 0.18 per cent. In other words no assimilating limit as regards glucose is to be found in normal persons.

Hansen terms this regulating mechanism the acceleration capacity of the organism, i.e., a capacity to accelerate the removal of the sugar from the blood. Justifiably she calls attention to the interesting fact that the maximum value to which the blood sugar rises has nearly the same value as that of the usual blood sugar threshold. This explains why in the majority of normal individuals we cannot

produce glycosuria by administration of carbohydrates. In the morning when the patient is fasting the blood sugar is about 0.09 to

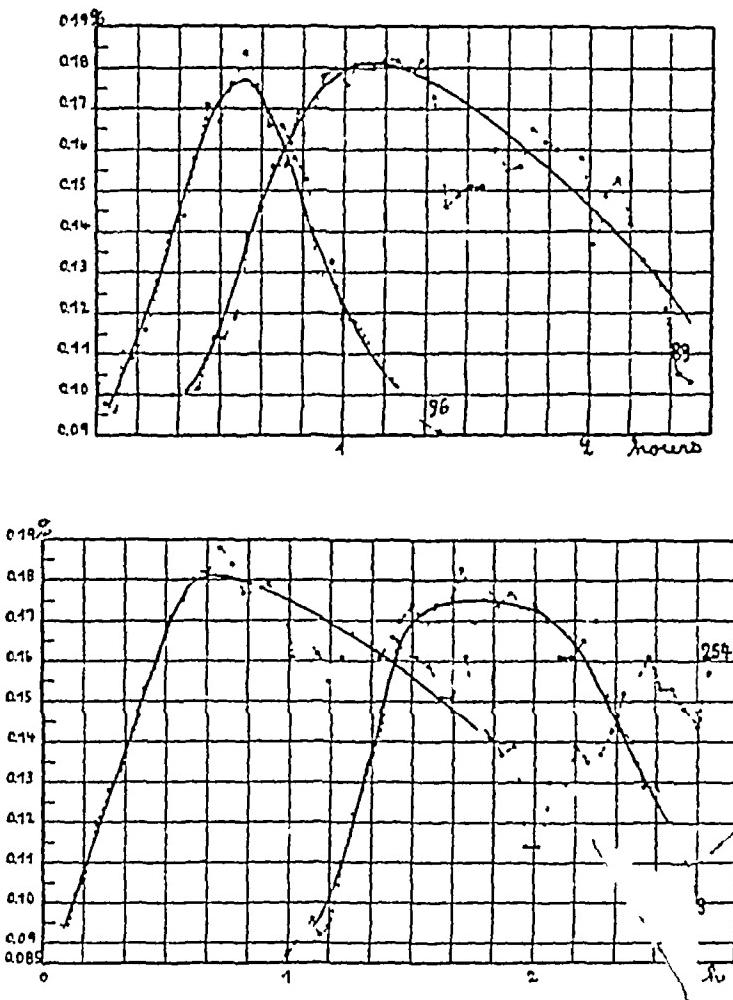


FIG 1 FOUR BLOOD SUGAR CURVES FROM A NORMAL INDIVIDUAL

- Curve 89 100 grams of glucose in 200 cc of tea
- Curve 96 20 grams of glucose in 160 cc of tea
- Curve 93 50 grams of glucose in 160 cc of tea
- Curve 254 200 grams of glucose in 250 cc of tea

0.11 per cent and after meals containing carbohydrates it rises to about 0.18 per cent, but not higher and does not exceed the threshold

Thus we see how the blood sugar regulation keeps the blood sugar value at a suitable level by means of two different mechanisms, (a) the blood sugar threshold, (b) the acceleration of blood sugar removal into the tissues

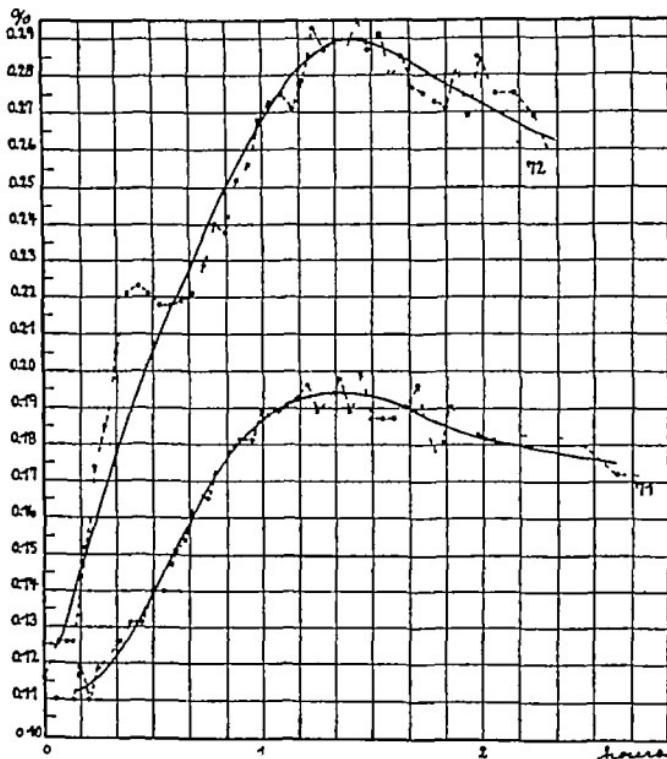


FIG 2 BLOOD SUGAR CURVES FROM A PATIENT WITH DIABETES MELLITUS

Curve 71 35 grams of glucose in 160 cc of coffee.

Curve 72 58 grams of glucose in 160 cc. of coffee.

When a disturbance in the blood sugar regulation occurs, glycosuria sets in. The classical example of this is seen in ordinary diabetes due to pancreatic insufficiency. Here hyperglycemia occurs, the organism having lost its power to remove the sugar from the blood when it exceeds the usual value. In the curves in figure 2 is seen

the blood sugar rise following the administration of glucose in a case of diabetes. The deficiency of insulin not only prevents the combustion and the hepatic storage but also the peripheral removal of glucose. Arterial and venous blood contain, as a rule, about the same quantity of sugar and hyperglycemia develops.

Glycosuria may occur, however, following the ingestion of glucose without the presence of diabetes. This may occur in two different ways. Either the threshold is lower than the normal, or the blood sugar after ingestion of carbohydrates may rise above the normal level. Both these possibilities deserve special study.

We have previously stated that the threshold is usually at 0.16 to 0.18 per cent. It lies, however, not infrequently higher, above 0.20 per cent or even higher still. It will readily be seen that a higher position of the threshold does not give rise to glycosuria and is consequently of little practical significance in normal individuals. It is a different matter when the threshold is subnormal. When the alimentary blood sugar rise is normal, the blood sugar may exceed the threshold and a glycosuria will result. In a certain number of patients the threshold is so low that the blood sugar always or almost always is above the threshold, we have then a case of true renal glycosuria. In such the threshold is found to be as low as from 0.05 to 0.12 per cent.

Should the threshold lie between 0.12 and 0.15 per cent we may encounter a renal alimentary glycosuria. Clinically this will manifest itself in what we have termed cyclic glycosuria. In the morning the urine is free from sugar, but after a meal containing carbohydrates the blood sugar rises during the day above the threshold, a glycosuria appears that again disappears in the evening or during the night.

It is significant to note that the position of the threshold may vary greatly in different individuals, but in the same individual the position is constant during his or her whole lifetime. This view as to the constancy of the threshold was first advanced by Faber and Norgaard (4) in 1919 at the Northern Congress for Internal Medicine at Copenhagen. This view was later confirmed as to normal individuals by Hagedorn. In respect to patients with constant or cyclic renal glycosuria, the continually recurring glycosuria alone shows that the

threshold is constantly low. We have also been able to demonstrate this fact time after time by means of examinations.

In this connection it is of importance to emphasize that very frequently renal glycosuria is a familial or hereditary weakness. We find a number of cases in the same family, who during their whole lifetime, suffer from renal glycosuria or a constantly recurring cyclic glycosuria. The low threshold is thus an individual characteristic, a constitutional abnormality and the glycosuria is not a sign of any metabolic disturbance. It is not the result of disease but is, as a rule, a congenital abnormality.

Whereas most students of this subject have come to agree with these views, opinions differ regarding the question of the position of the threshold in true diabetics. Here the opinion has been constantly advanced that the threshold is very mobile and is especially displaced under the influence of glycosuria and under the influence of a treatment causing the glycosuria to subside.

In 1915, the Swede, Engstrand maintained this and was of the opinion that when a decrease in the glycosuria was brought about by dieting, the blood sugar threshold rose in value and the carbohydrate tolerance in diabetics was also improved. From this point of view it will be observed that the question of the constancy of the threshold in diabetics is of great practical significance. Hamman and Hirschman (9), in 1917, likewise came to the conclusion that they could demonstrate that glycosuria caused a displacement of the threshold and Williams and Humphreys (16) obtained a similar result. In Scandinavia, too, similar views have been repeatedly advanced.

In contrast to these ideas, we have been led by our investigations carried out with Norgaard (4) and Hansen, (3) to conclude that in diabetics also the blood sugar threshold is constant in every individual and that it is influenced neither by glycosuria nor by the duration or degree of the affection. Just as in non-diabetics the threshold is in some diabetics at the normal level, but, as we have found in a number of cases, it may be either higher or lower. In any given case however it is always found at the same level.

The reason that this view has not been generally accepted must be attributed to the fact that the difficulties inherent in an accurate

determination of the threshold, and the means of surmounting these difficulties, have not been realized.

The method frequently employed has been to determine the value of the blood sugar in the morning while the patient is still fasting and to compare this value with an examination of the urine passed later. If the whole of the urine passed during the following day is used for this purpose it will readily be seen that this will lead to considerable error, as the blood sugar in the course of the day rises after every carbohydrate-containing meal. I am of the opinion that Wilhams and Humphreys and later Petrin were led to erroneous conclusions by such a procedure. The determination of the threshold by this means will give a value that is too low. The same error occurs also if the morning blood sugar is compared with the urine passed immediately after the blood sample is withdrawn. This urine actually corresponds to the blood sugar values of a considerably earlier point of time than when the urine was passed. As the blood sugar has further diminished in the morning, too low a blood sugar value is compared with the urine examined. Thus by this means too low a value for the threshold is obtained, and a varying and unknown error is produced.

A better method is to determine the blood sugar curve after an alimentary ingestion and to observe at what blood sugar level the glycosuria sets in and ceases. Here, too, however, there are important factors to take into consideration. In order to ascertain the highest peak of the blood sugar curve it is necessary to make very frequent blood sugar determinations, best every five minutes. Then again venous blood must not be used, but cutaneous blood, that is to say, arterial blood. It is, as a matter of fact, the blood sugar percentage in the renal arteries that must be presumed to be a decisive factor in the occurrence of the glycosuria. For such serial determinations, Hagedorn and Normann Jensen's (8) method for micro-determinations is more suitable than any other. The whole of the blood is here used in the analysis. It would perhaps be more correct to determine the content of the plasma, but with the small quantities of blood one is obliged to use for serial determinations at very short intervals of time, this cannot be done.

When the blood sugar curve is determined in this manner, it is

observed that the glycosuria sets in late during the rise in blood sugar, usually not until the threshold is overstepped, whereas it lasts during the fall of the blood sugar for a long time after the glycemia has passed its maximum and after the threshold value has been passed during the fall of the blood sugar to normal values (fig 3). Hamman and Hirschman (9) in 1917 were the first to call attention to this phenomenon, and they therefore came to the conclusion that glycosuria depressed the threshold. We found, however, that it depends upon a measurable time elapsing from the moment the threshold is overstepped until the glycosuria is established. The time varies some-

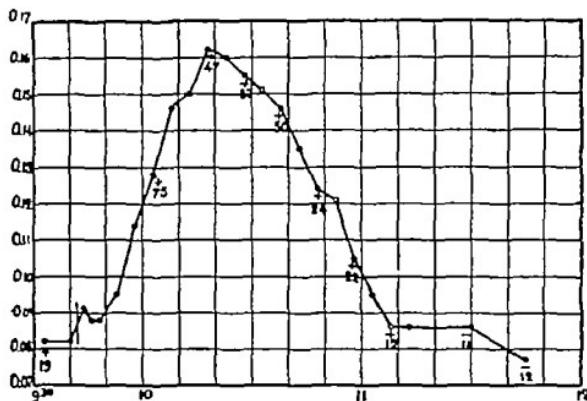


FIG 3 GLYCOSEURIA 50 GRAMS OF GLUCOSE AT 9:42 A.M., FEBRUARY 26, 1923  
+ = glycosuria, - = no glycosuria. The figures indicate the amount of urine

what in the different experiments and can by no means be explained by the passage of the urine through urethra and bladder. Consequently, to determine the threshold we must have two blood sugar curves, determined by serial examinations after administration of carbohydrates. If the one administration does not cause glycosuria, whereas it sets in after a larger administration, it is evident that the position of the threshold is between the peaks of the two curves. The two curves in figure 4 from a diabetic serve as an example. After 25 grams of glucose were administered the blood sugar curve rose to 214 mgm per cent without glycosuria. After 35 grams of glucose to 236 with glycosuria. Thus between these two values lay the

glycosuria threshold, although during the last test glycosuria was still present at a blood sugar value of 205. This curve (fig 5) demonstrates the necessity for very frequent measurements. Every five minutes a test is taken. There is a high peak between two tests.

By a series of experiments carried out with this accurate method we found the threshold to be constant in diabetics. By repeated

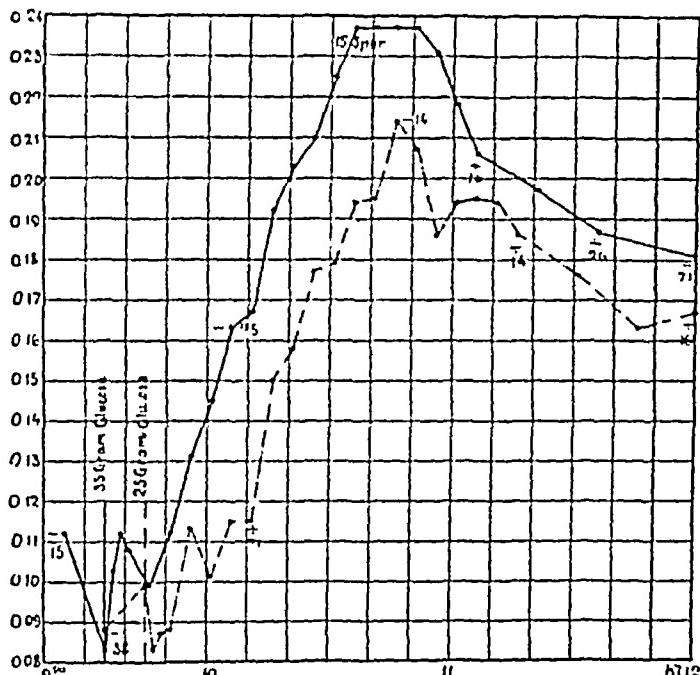


FIG. 4 DIABETES MELLITUS

Broken line, blood sugar curve after 25 grams of glucose on December 13, 1922. Solid line, blood sugar curve after 35 grams of glucose on December 18, 1922.

examinations during the course of treatment we found similar values, even if the patient by dieting was kept free from glycosuria during the whole time, and under conditions which according to earlier views would cause a rise in the threshold.

To elucidate this the examples in table 3 are given.

In each of these cases the threshold is determined by using two blood sugar curves. Between the lower and the higher value is the

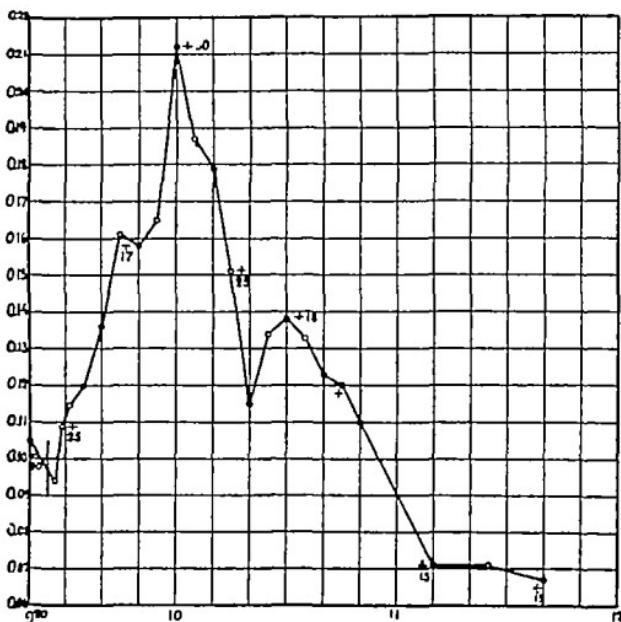


FIG 5 GLYCOSURIA BLOOD SUGAR CURVE AFTER 50 GRAMS OF GLUCOSE MARCH 10, 1923

TABLE 3

	Age	Duration of diabetes	Situation of threshold (mgm. per cent)	Interval
A. H.	24	1½ years	August 30-31 1921, over 169, below 192 September 14-15 1921 over 171 below 179	2 weeks
H. M	22	8 days	November 15-16 1921, over 191, below 205 December 5-6 1921 over 195 below 214	3 "
N P M	35	4 months	December 13-14 1921 over 160 below 182 January 20-21 1922, over 162 below 169	5 "
M H	16	9 months	May 14-21 1921 over 169 below 190 October 15-16 1923 over 160 below 171	2½ years

position of the threshold. The determination is repeated after a certain time and after the patient has been kept free from glycosuria for two to five weeks by regulation of the diet. In the last case the

patient was investigated two and a half years later and the threshold was found to be still at about 170 mgm.

We believe therefore that it may be maintained that the glycosuric-threshold in diabetics, just as in non-diabetics, has a constant value in one and the same individual, whereas in different individuals it may vary somewhat. The value is independent of the duration of the disease or the age of the patient.

As a general rule we must conceive the glycosuric threshold as an inherent quality in the individual, a constitutional quality that is constant as regards every single individual, but may vary considerably from individual to individual in diabetics as in normal persons. By this we do not infer that the sugar threshold of an individual does not change under varying conditions. Thus we know that experimentally, glycosuria without hyperglycemia may be produced by administration of phloridzin and some few other substances. A change of threshold may also cause the frequent glycosuria of pregnancy.

Since the investigations of Maase, Novak, Porges and Strisower, Frank and Jacobsen we know that glycosuria frequently occurs in pregnancy with normal blood sugar, and the question of glycosuria of pregnancy subsequently has been the object of a series of researches, especially by Frank (5) (6) and his collaborators, by Holst, Fr. Jensen and many others. The diagnostic value of alimentary glycosuria has been studied especially in early pregnancy (Frank). It would appear that the diagnostic value is rather insignificant, as an alimentary glycosuria may so often be observed in non-pregnant women. The phenomenon itself, however, is of great significance in studying the threshold problem.

In order to ascertain whether the glycosuria of pregnancy is exclusively due to a fall in the threshold, and whether such a fall is purely a transient disturbance, I have made accurate threshold determinations during pregnancy and after parturition in two women, with the following results:

1 A 20-year-old primipara had a normal delivery on January 10, 1924. Before parturition, on December 20 and 27, 1923, the threshold was over 121 and under 132 mgm while after parturition, on January 18, 1924, the threshold was under 156 mgm and on August 12, 1924, the threshold was over 197.

2 A 19-year-old primipara had a normal delivery on February 9, 1924. Before

parturition on February 6 and 8, 1924, the threshold was over 95 and under 131 while after parturition, on February 15, 1925, it was under 142 mgm and on April 9, 1925, it was over 150 mgm.

In both patients during pregnancy glycosuria was demonstrated on ordinary diet, but their urine was free from sugar in the mornings. In

TABLE 4  
*Before parturition*

December 20 1925 Kl 9 10 Indg. 25 grams glucose			December 27 Kl 9 20 15 grams glucose				
Time	Blood sugar	Urine		Time	Blood sugar	Urine	
		Dihreals	Sugar			Dihreals	Sugar
	mgm per cent	cc.			mgm. per cent	cc.	
9 02	54	23	—	9 12	64	50	—
10	54				19	64	
13	61				22	79	
15	70				24	77	
17	81				26	80	
22	104	27	—		31	107	
27	114				36	109	
32	114	37	—		41	121	
37	121				46	117	
42	132	30	+		51	100	
47	121				56	95	
52	111	14	+	10 01	73	42	
59	93				06	61	
10 02	77	12	+		11	55	
07	72				16	53	
12	61				21	55	
17	59				26	55	
22	52	13	+		31	53	
27	52				35	55	
32	46	8	—			44	
37	43						
42	48	20	+				

both cases the threshold before parturition lay below 132 and some months after in case 1 above 197, in case 2 above 150 mgm. The normal value of the sugar threshold was thus again reached and the glycosuria had disappeared. It will be observed that the normal threshold was not reached immediately after parturition, for during the first weeks the threshold was still abnormally low. Here we have

therefore a striking example of the fact that the threshold, under certain abnormal conditions may be temporarily changed.

In order to give further details of these determinations of threshold, the blood sugar analyses for the 4 leading tests made in case 1 will be given (See tables 4 and 5 and figs. 6 and 7)

TABLE 5  
*After parturition*

January 18 1924 Kl 9 10 25 grams glucose				August 12, 1924 Kl 9 35 100 grams glucose			
Time	Blood sugar	Urine		Time	Blood sugar	Urine	
		Diuresis	Sugar			Diuresis	Sugar
	mgm per cent	cc			mgm per cent	cc	
9 00	81	22	—	9 25	94	19	—
10	78			31	108		
13	78			37	96		
15	83			39	108		
17	83			41	120		
22	103			46	140		
27	125	10	—	51	169		
32	156			56	190		
37	144			10 01	197		
42	154	12	—	06	192		
47	127			11	187	40	—
52	131			16	174		
57	110	14	Trace	21	174		
10 02	110			26	183		
07	88			31	165	55	—
12	94	14	+	36	165		
17	85			41	156		
22	88			46	174		
27	85	16	Trace	51	103		
32	70	14	—	56	156		
37	74			11 01	152		
	74			06	152		
				15	140	50	—

Having discussed the disturbances in the blood sugar regulation produced by abnormal conditions of the blood sugar threshold, we shall now consider the results of disturbances in the second regulating mechanism, namely the removal of the blood sugar in alimentary hyperglycemia. We have mentioned disturbance of this function found in true diabetics. But disturbance in this regulating mech-

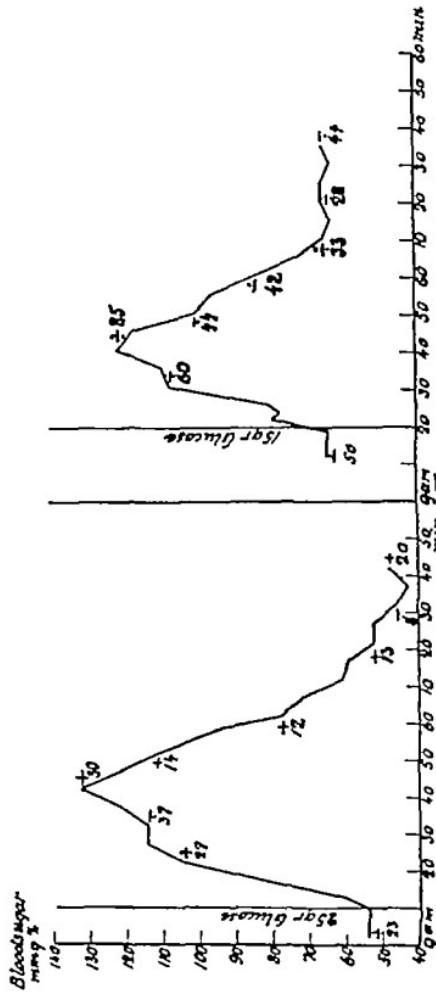


FIG. 6 BLOOD SUGAR CURVES BEFORE PARTURITION

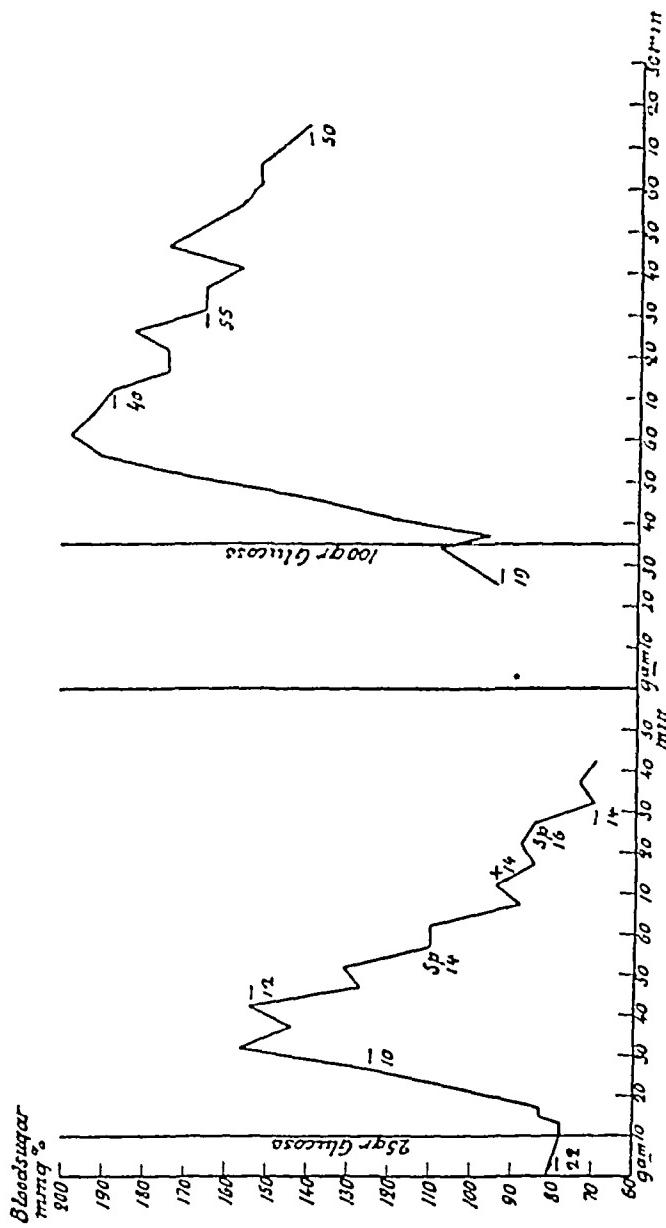


FIG 7 BLOOD SUGAR CURVES AFTER PARTURITION

anism may be observed without there being any question of diabetes and without there appearing to be a deficiency of insulin production

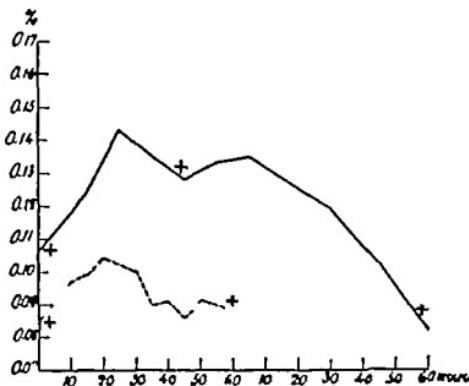


FIG 8 TRUE RENAL DIABETES—CONSTANT GLYCOSURIA  
Blood sugar threshold below 90 mgm.

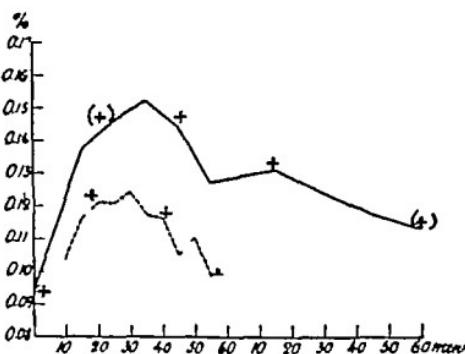


FIG 9 CYCLIC GLYCOSURIA, THRESHOLD BELOW 120 MGML

This disturbance manifests itself by an abnormal rise of the blood sugar after an alimentary ingestion. Instead of stopping at about 180 mgm as is the case in normal individuals, the blood sugar level

rises up to between 200 or 300 mgm and if the blood sugar threshold is at its usual position it is exceeded and glycosuria sets in

The curves in figures 8 to 12 will demonstrate these different cases

It will be readily understood that if an unusual rise in blood sugar occurring after alimentary ingestion is the only abnormality, a cyclic glycosuria will appear clinically in quite the same manner as the cyclic glycosuria that occurs with a low blood sugar threshold. In the morning the blood sugar is normal and the urine free from glucose. After

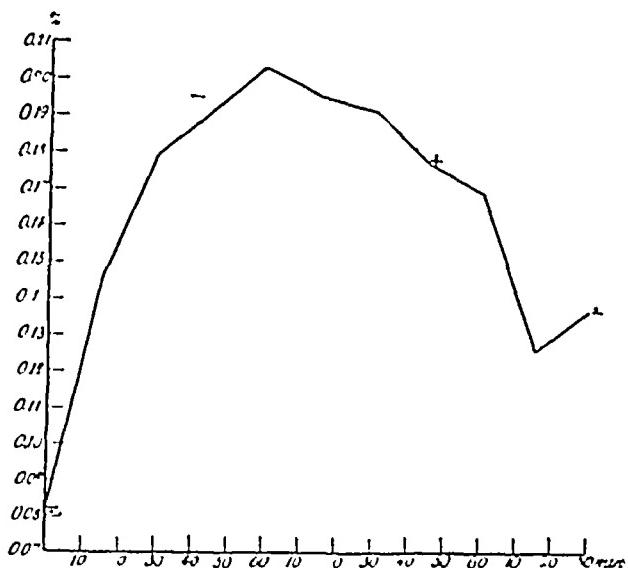


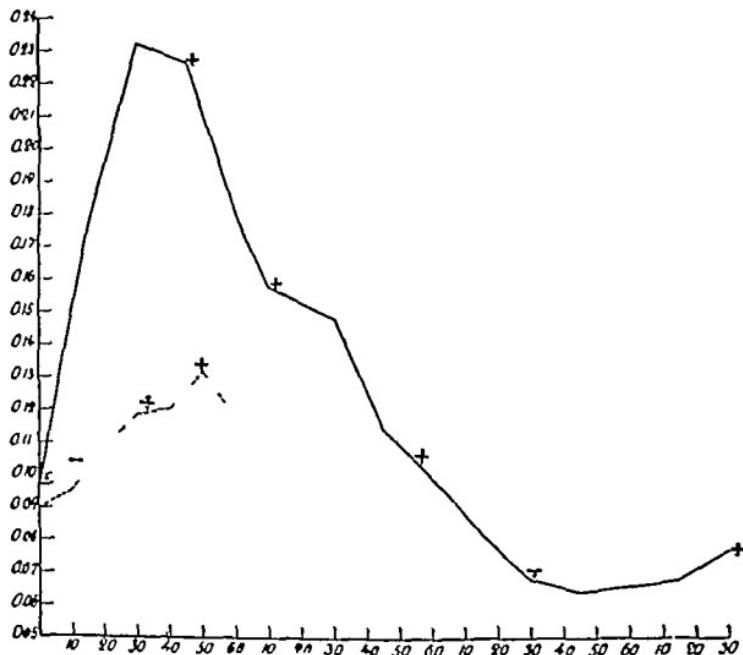
FIG 10 CYCLIC GLYCOSURIA AFTER THE INGESTION OF GLUCOSE, THE CURVE RISING ABNORMALLY HIGH TO 200 MGm AND ABOVE THE THRESHOLD

meals the blood sugar rises abnormally high, exceeding the threshold. We then get glycosuria that again disappears during the evening and night. The same can be observed in incipient, mild diabetes, but doubtless such a cyclic glycosuria, which is due entirely to an excessive alimentary rise may be observed fairly frequently and may be present for years, perhaps forever, without the development of true diabetes.

In the examination of 163 patients who formerly had been declared by life insurance companies to be diabetics on account of a glycosuria, Holst (12) was able to demonstrate the frequency of this benign form

of glycosuria. Such a demonstration is obviously of great practical significance to the patient.

He found 27 cases of this type of glycosuria which have been observed for from 1 to 25 years without any other sign of diabetes having manifested itself, although the majority of them have ceased restricting their diet. By repeated examinations of the same patient he has demonstrated that year by year the same abnormally high rise in



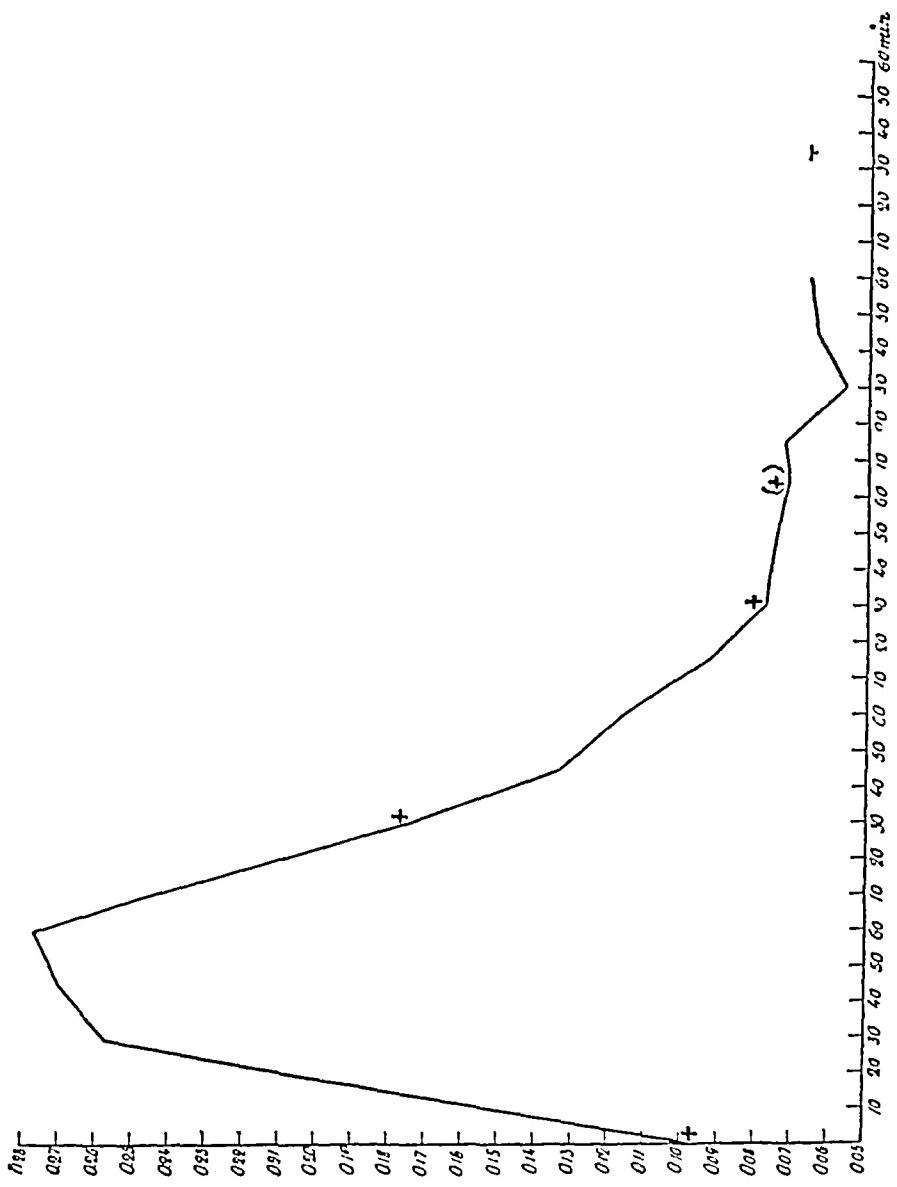


FIG. 12 A COMBINATION OF A HIGH ALIMENTARY RISE AND A LOW THRESHOLD  
 In this case the threshold is so low that there is a nearly constant renal glycosuria. The threshold must be below 100 mgm.

several cases Holst found that the high alimentary rise later disappeared

It is readily seen that this form of cyclic benign glycosuria is more difficult to distinguish from a beginning diabetes than a glycosuria due only to a low threshold. A mild diabetes would manifest itself in the same way as a cyclic glycosuria if the diet is restricted.

The question is whether it is generally possible for us to distinguish between these cases and a mild true diabetes when faced with a cyclic glycosuria that is not due to low threshold.

In this respect Holst (13) has called attention to the fact that when a patient lives on a full normal diet without carbohydrate restriction, an absolutely normal fasting blood sugar will almost certainly preclude true diabetes. Should a cyclic glycosuria set in during the course of the day an abnormally low threshold or an excessive alimentary rise is indicated, neither of which denote an abnormal metabolism which has any serious influence on the future of the individual.

This conception cannot as yet be considered so sufficiently established that it can be carried into practice but the conception deserves to be carefully tested.

As a result of the consideration we have given to the question of glycosuria we wish to emphasize the point that two essentially different forms of glycosuria exist. The first is due to a disease of the islands of Langerhans. This is true diabetes. The second form of glycosuria is due to a deficient blood sugar regulation which is dependent either on an habitual abnormally low threshold or on an habitually high alimentary blood sugar rise. In some cases both may be observed, the patient presenting both an abnormally low threshold and an abnormally high alimentary blood sugar rise. In 75 such cases observed for from 1 to 25 years Holst (11) found the cause to be an abnormally low threshold in 22, an excessively high alimentary rise in blood sugar in 15 and in 11 a combination of low threshold and high alimentary rise. In 27 the type was not determined. In all these cases the glycosuria is the result of an individual constitutional abnormality rather than of disease. This form of glycosuria is harmless to the individual and deserves to be estimated as such by doctors and insurance companies.

The practical significance of knowledge of these benign glycosurias

cannot be overestimated. The more extensive and the more careful the examination of urine for glucose becomes among medical men the more frequently will a glycosuria be discovered and the more frequently will individuals be regarded as diabetics in spite of the fact that they are only suffering from an insignificant passing glycosuria. This very often occurs. At the aforementioned examination of 163 applicants for life insurance conducted by Holst, only 30 per cent were true diabetics, the remainder suffering from benign glycosuria.

When all the samples of urine from patients are examined in a routine manner after they have partaken of a meal containing much sugar, as in Denmark, for instance after sweet soup, one is surprised to note that glucose may be demonstrated in one or more samples of urine in 20 to 30 per cent of the patients. After ingestion of larger quantities of glucose considerable glycosuria is found in about 33 per cent, and even after the ingestion of starch it may be seen in about 20 per cent. A daily recurring cyclic glycosuria on ordinary diet is of course less frequent.

The correct interpretation of a glycosuria is of the very greatest importance to the patient. In making the *diagnosis* there is a possibility of error in two directions. A harmless excretion of sugar may be regarded as an incipient diabetes mellitus, an error which may have economic, social, and of not least import, psychic consequences of a grave nature for the patient. It will be just as unfortunate if early diabetes is regarded as benign glycosuria, for the mistake in such cases is often not discovered until the disease has progressed and is perhaps already so far advanced that the most appropriate time to attack and arrest its development has passed.

The difficulty arises when we detect a glycosuria without other symptoms of diabetes. The subjective symptoms may be entirely absent in true diabetes. Among the applicants for life insurance, Holst found that of 43 diabetes cases fortuitously detected at the first observation by glycosuria only 6 presented typical symptoms of diabetes, while 33 or 77 per cent showed complete absence of any subjective sign of the disease. It is therefore necessary to make blood sugar examinations in every case of glycosuria which is not of a purely transitory nature. What we want to know is if there is any hyperglycemia when the patient is fasting.

Most of the cases of glycosuria are discovered by the general practitioner, who, at present is not in a position to make the necessary blood examination. Some time will usually elapse therefore from the time the glycosuria is discovered until it comes to be investigated. It is of great importance for this investigation that no dietetic treatment is begun in the interval, otherwise there is a strong possibility of erroneous conclusions, because an existing hyperglycemia may disappear.

If the patient is examined without previous treatment the diagnosis will depend upon the fasting blood sugar. If this is abnormally high the diagnosis of true diabetes should be made. If it is normal, the examination ought to be repeated several times at intervals of a few weeks. In the intervening period the patient should be allowed to eat food with carbohydrates.

If the patient has shown for a long time a normal fasting blood sugar, that is below 0.11 per cent, although the food is rich in carbohydrate, it can be taken as highly probable that the glycosuria is of benign nature and is not indicative of diabetes.

As a supplementary investigation the determination of the type of glycosuria may be carried out. This is done by obtaining several blood sugar curves after administration of carbohydrates. It is of most interest when it can be demonstrated that a low threshold is the *only* cause of the glycosuria. Then the case must be considered as certainly benign.

If there is an alimentary rise to abnormal height more caution is necessary. We see this in diabetes but, as mentioned above, a cyclic glycosuria due to excessive alimentary rise may be present for years without the development of diabetes.

Regarding these observations the question may be raised as to whether an individual who shows a benign glycosuria is more apt than other people to develop later a true diabetes. This is said by several authors to occur and it is difficult to deny. I can only say that I do not know of any case where a benign glycosuria has turned out to be true diabetes after it was definitely determined to be a benign glycosuria.

If we glance back at the development of the doctrine of benign glycosuria during the last decennium it will be seen that it is due to the careful study of the glucose content of the blood and that

these researches have been especially aided by the micro method introduced by the Norwegian, Ivar Bang, by which the sugar content of blood can be determined from a few drops of blood. Elaborated in the micro method of Hagedorn and Norman Jensen, it gives us a means of making accurate blood sugar curves after alimentary ingestion. The whole development is a fine example of the advantage of exact laboratory analyses, and in addition it demonstrates the fact that a full understanding of the conditions can only be obtained by the use of analyses on a large number of patients, and that only by accurately studying the patients over a considerable period of time is the significance of deviations from the normal to be seen.

#### BIBLIOGRAPHY

- 1 Faber, K Wien klin Woch, 1923, lxxiii, 1190 Bestimmung der Blutzuckerschwelle bei der Glykosurie
- 2 Faber, K Hospitalstid, 1925, lxviii, 1039 Glycosuric Blood Sugar Threshold in Diabetics and in Pregnants
- 3 Faber, K, and Hansen, K M Acta Med Scandinav, 1923, lviii, 372 Threshold of Glycosuria and Errors Involved
- 4 Faber, K, and Norgaard Acta Med Scandinav, 1921, lii, 670 The Relations Between Glycosuria and Glycaemia
- 5 Faber, K, and Norgaard Acta Med Scandinav, 1921, lii, 289 Studies on the Threshold of Glycosuria
- 6 Frank, L Arch f exp Path u Pharm, 1913, lxvi, 387 Über experimentale und klinische Glykosurien renalen Ursprungs
- 7 Frank, L, and Nothmann, M Münch Med Woch, 1920, lxvii, 1433 Ueber die Verwertbarkeit der Renalen Schwangerschaftsglykosurie zur Frühdiagnose der Schwangerschaft
- 8 Hagedorn, H C Blodsukkerregulationen hos Mennesket Copenhagen, 1921 Also Physiological Papers dedicated to Prof Aug Krogh Copenhagen, 1926, 80
- 9 Hagedorn, H C, and Jensen, N Ugesk f Laeger, 1918, lxxx, 1217 Test for Sugar
- 10 Hamman, L, and Hirschmann, I I Arch Int Med, 20, 761 Studies on Blood Sugar I Alimentary Hyperglycemia and Glycosuria as a Test of Sugar Tolerance
- 11 Hansen, K M Investigations on the Blood Sugar in Man Copenhagen, 1923
- 12 Holst, J E Zeit f klin Med, 1922, xciv, 394 Studien über die Alimentaire Glykosurie

- 12 Holst, J E Acta Med Scandinav , 1922, lvii, 188 Untersuchungen über leichte Glykosurien
- 13 Holst J E Acta Med. Scandinav , 1925, lxiii, 47 Investigations into Benign Glycosuria and Diabetes Mellitus.
- 14 Jacobsen, A T Biochem Zeitschr , 1913, lvii, 446
- 15 Jacobsen, A T Blodsukkerindholdet hos Normale og ved Diabetes Mellitus. Copenhagen, 1917
- 16 Williams, J R., and Humphreys E M Arch. Int Med., 1919, xxiii, 537 Clinical Significance of Blood Sugar in Nephritis and Other Diseases



## STUDIES ON RED CELL DIAMETER

### I IN HEALTH AND IN PERNICIOUS ANEMIA

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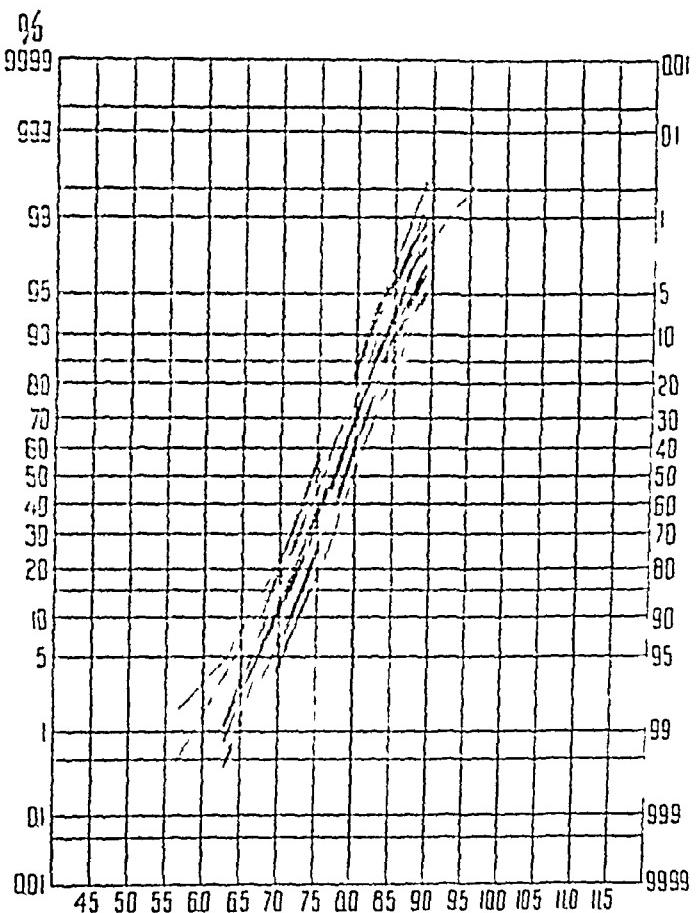
During a visit to Guy's Hospital in the summer of 1924 one of us (J. H. M.) became interested in Hurst's (1) use of Price-Jones' method (2) of measuring the diameter of the red cells of the blood in cases of pernicious anemia, and was impressed not only with the possible importance of this procedure as a means of differentiating clinical types of anemia, but also with its possibilities as a method of investigating many problems in hematology. Accordingly measurements of this kind were promptly started at the Massachusetts General Hospital and have been in progress ever since. At the present time we are ready to report on a series of 20 normal persons and one of 25 cases of pernicious anemia.

#### METHOD

The method employed in this study is as follows. In a film of blood stained by Wright's stain 250 red cells are measured in their greatest horizontal diameter by means of an ocular micrometer which has been calibrated for a microscopic tube length of 170 mm. by the use of a stage micrometer. With a mechanical stage it is easy to move the cells from right to left only those falling directly beneath the scale are measured. With our scale it is possible to measure to every  $0.66\mu$  which is sufficiently accurate for the desired purpose. As it is possible that altitude, and perhaps other geographic factors may influence the size of red cells, it should be noted that all of the observations made by us were done in Boston, Massachusetts.

As the measurements are made a tally sheet is kept. When the desired total has been obtained, curves of either the frequency or summation type may be constructed from the data. Price Jones (3) has used both types, but usually the former. We have rather preferred the latter, especially when plotted on Whipple's arithmetic probability paper, the advantage of it being that such a curve is

more readily smoothed and lends itself more easily to comparing data of different observers, regardless of the number of cells measured or the size of the unit of measurement.



### DIAMETERS IN MICRONS

FIG. 1 CURVES OF DISTRIBUTION OF RED CELL DIAMETERS IN 20 NORMAL PERSONS, 10 MEN AND 10 WOMEN

#### RESULTS IN NORMAL PERSONS

Films of blood from 20 normal persons between twenty and thirty years of age, 10 men and 10 women, were measured as described above. The curves obtained are shown in figure 1. Plotted on arithmetic

probability paper any natural distribution yields approximately a straight line. Reference to figure 1 will show that the diameters of the red cells in our 20 normal subjects not only conform very closely to such a law, but also show a marked parallelism among the several members.

TABLE 1  
*Normal persons*

Subject	Sex	Double dispersion (84 - 16 percentile)	Median 50 percentile grade	Mean
		microns	microns	microns
C T	F	1 1	7 7	7 6
M. M	F	1 0	7 7	7 7
V B	F	1 2	7 9	7 9
H G	F	1 1	8 0	8 0
F P	F	1 1	7 7	7 7
G D	F	1 1	7 9	7 8
M R	F	1 1	7 7	7 7
J McI	F	1 1	7 5	7 4
M D	F	1 1	7 5	7 5
F K. T	F	1 1	7 7	7 7
W B	M	1 0	7 8	7 9
C M J	M	1 1	7 5	7 6
W O T	M	1 2	7 8	7 7
D B D	M	1 0	7 9	7 8
D R. H	M	1 1	7 7	7 7
C W H.	M	1 1	7 5	7 5
J S L	M.	1 1	7 6	7 6
C I K	M	1 2	7 4	7 4
S O	M	1 1	7 7	7 7
G W T	M	1 5	7 5	7 5
Average { Women		1 1	7 7	7 7
	Men	1 1	7 6	7 6
Grand average		1 1	7 7	7 7

For purposes of comparison with pathologic bloods, or with normal bloods under unusual circumstances, we have, at the suggestion of Prof E B Wilson, used the median (fifty percentile grade), and the sixteen and eighty-four percentile grades as taken from each individual curve. The median gives us the center of the range of dis-

tribution of the red cell diameters, and the difference between the sixteen and eighty-four percentile grades gives double the dispersion. That is to say, in any given case the former shows whether the cell population as a whole tends to be of greater or less diameter than that of the usual normal, while the latter gives us a quantitative estimation of the degree of anisocytosis. The values of these functions for our series of normals are given in table 1, together with the mean diameter, this last being the function used by most other investigators. The medians and dispersions were read off from smoothed curves, the means were calculated from the tally sheets.

The table brings out several interesting points. First, with regard to the median we observe that the extreme variations in this function for the entire series is less than plus or minus 4 per cent of the average. Second, we note that between the sexes there seems to be no difference of importance, and third, between the medians and means of all there is a very close similarity, in fact the grand average for each of these functions is the same. The dispersions too show a high degree of consistency, with the single exception of G W T the extreme variation being within plus or minus 9 per cent of the average for the entire series. Here again there is no significant difference between the sexes.

The object of this study of the blood of healthy persons, as already indicated is to establish norms. To that end it would be desirable to plot frequency curves of the medians and dispersions, but the present series is not large enough to permit of that. It may be said, however, that measurements of the red cell population of 20 normal persons show a high degree of similarity, and that, in round numbers, the normal range for the median is between 7.4 and 8.0 microns, and for the dispersion 1.0 to 1.2 microns. Until such time as a large series of normal data is obtained, the foregoing will serve very well for normal values.

The extreme variation in red cell diameter should also be noted in this series of twenty normals the smallest cell observed was 5.3 microns, the largest 9.9 microns.

## RESULTS IN PERNICIOUS ANEMIA

Data similar to those on normal persons have been collected on 25 cases of undoubted pernicious anemia. All the individual curves

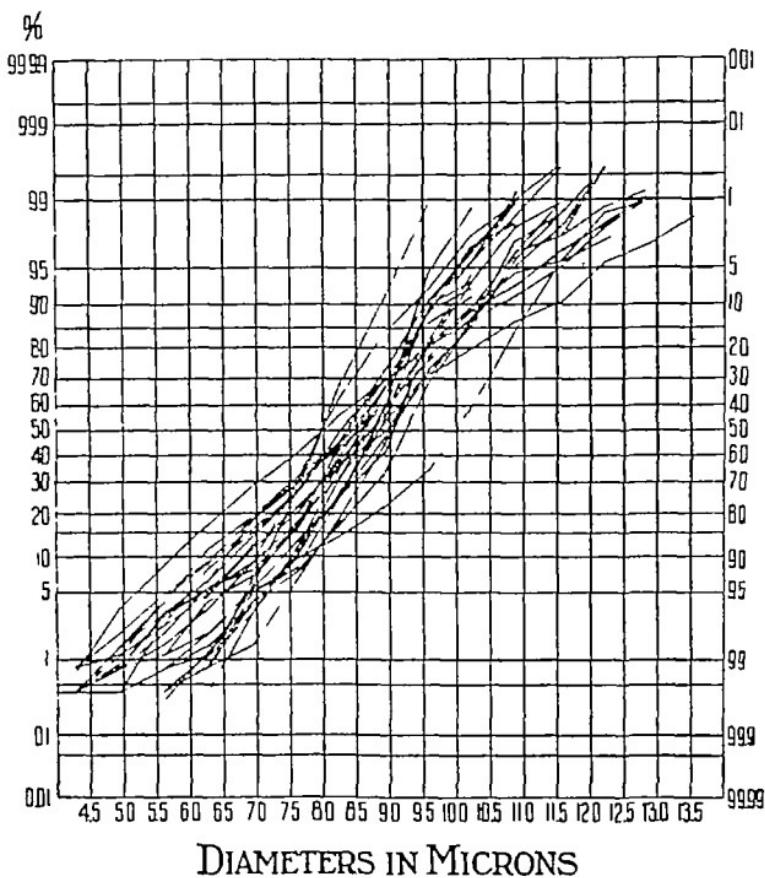


FIG. 2 CURVES OF DISTRIBUTION OF RED CELL DIAMETERS IN 25 CASES OF PERNICIOUS ANEMIA

are shown in figure 2, and the readings taken therefrom in table 2. Several striking departures from the normal series are obvious. For instance, not only the dispersion is greater (anisocytosis), but also the median definitely so. The average for the median in the 25

pernicious anemia cases is 8.6 microns with a maximum of 10.0 microns (16 per cent above the average) and a minimum of 8.0 microns (7 per cent below the average). The average dispersion is

TABLE 2  
*Pernicious anemia\**

Patient	Double dispersion (84 and 16 percentile)	Median 50 percentile grade	Mean
	microns	microns	microns
1	2.5	8.6	8.3
2	3.2	8.5	8.4
3	2.9	8.3	8.2
4	2.4	10.0	9.8
5	2.9	8.4	8.5
6	1.4	8.7	8.6
7	2.9	8.3	8.5
8	2.5	8.4	8.3
9	2.3	8.9	8.9
10	1.7	9.0	8.9
11	3.1	9.0	9.0
12	1.5	8.8	8.8
13	1.8	9.2	9.2
14	1.7	9.0	8.9
15	2.6	8.8	8.7
16	1.8	8.7	8.6
17	1.4	8.0	8.0
18	2.8	8.7	8.7
19	2.5	8.7	8.5
20	3.6	8.0	7.9
21	1.7	8.0	8.2
22	1.6	8.6	8.6
23	2.1	8.6	8.4
24	1.8	8.5	8.4
25	1.7	8.4	8.2
Average	2.3	8.6	8.6

\* Curves smoothed when possible

2.3 microns with a maximum of 3.6 microns (plus 57 per cent) and a minimum of 1.4 (minus 39 per cent).

The mean in individual cases varies somewhat more from the median than in normal persons, but not to any significant degree. The greater divergence is due of course to the fact that these pernicious

anemia curves are less symmetrical than those of the normal persons. In all but four instances it was not possible to smooth them, and the actual plot was used in taking off the readings.

The largest red cell found in pernicious anemia was 13.9 microns, the smallest 4.0 microns.

#### DISCUSSION

Since the discovery of the human red cells by Leeuwenhoek in 1673, measurements of their diameter have been made by numerous observers, their findings for the average diameter in normal blood ranging from 3.01 microns to 14.9 microns, with the major part of the observations for both wet and dry preparations lying between 7.5 and 8.0 microns. The literature on the micrometry of blood is so extensive and presents so many different methods of investigation that a separate paper will be devoted to its history.

However, in connection with our findings in normal blood, it seems fitting in this paper to call attention to the work of three other observers—one of fifty years ago, Richardson, the others of the present time, Ohno and Gievius. Richardson (4) in 1877 at the centennial celebration in Philadelphia obtained blood smears from representatives of fourteen different nationalities and from them made red cell diameter measurements. Fortunately in reporting his data he gives, besides the mean, two different percentile grades so it is possible to construct curves on arithmetic probability paper comparable to our own. Such curves for his findings are shown in figure 3. The similarity among the individual curves is marked, the medians ranging from 7.76 microns to 8.01 microns, and the dispersions from 0.57 microns to 0.77 microns. The former are in very close agreement with our findings, the latter are somewhat lower than ours, the fact that Richardson gives only two percentile grades from which we can construct curves might possibly be a factor in causing this difference. By this investigation Richardson has shown quite conclusively that there is apparently no significant difference in the bloods of different nationalities as far as red cell diameter is concerned.

In 1925 Ohno and Gievius (5) published their results of a very careful study on the diameter of human red blood cells. First, they measured red cells from four individuals, both in plasma and in dried

smears, and found no significant difference between the two, the average for the diameter in plasma being 7.98 microns and in the smears 7.99 microns. Having found no difference in diameter between

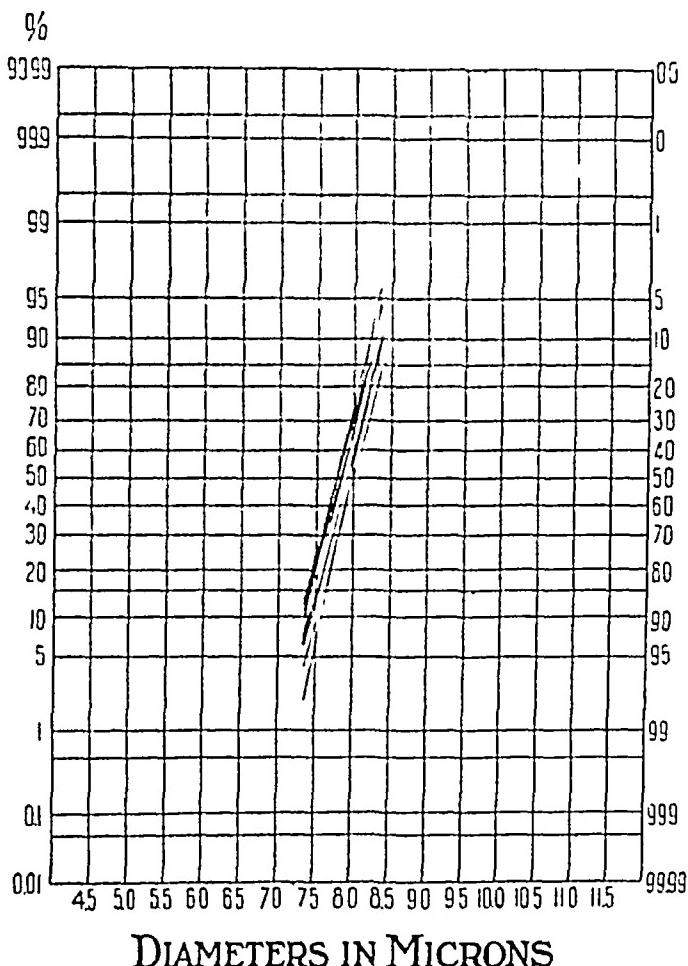


FIG. 3 CURVES OF DISTRIBUTION OF RED CELL DIAMETERS IN INDIVIDUALS FROM FOURTEEN DIFFERENT NATIONS

(From Richardson, 1877)

the wet and dry method they then measured red cells in dried smears from 14 normal persons, 7 men and 7 women, reporting their results in tabular form showing the per cent of red cells lying within each

0.25 micron from 6.00 microns to 9.99 microns. These data can be plotted on arithmetic probability paper giving the curves shown in figure 4. From these curves it will be seen that the median diameters

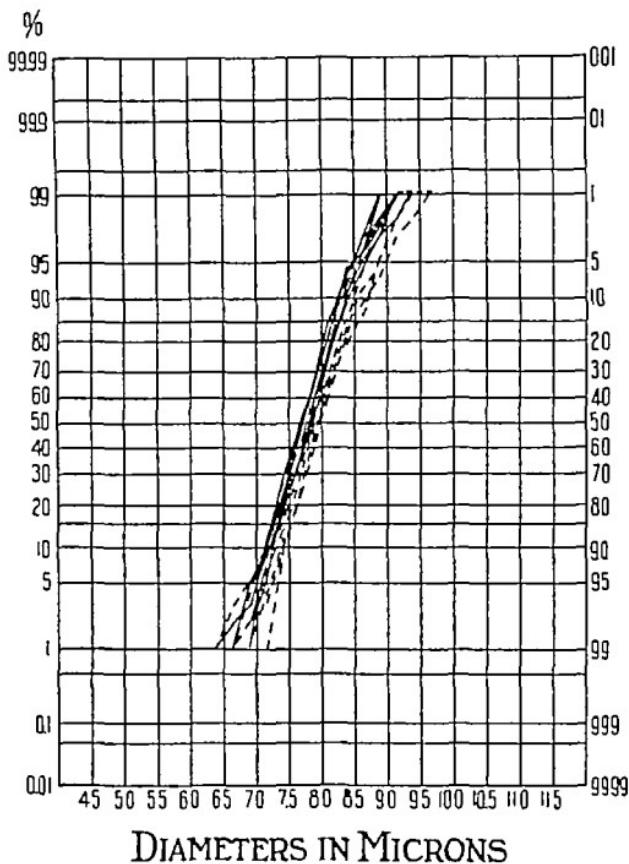


FIG 4 CURVES OF DISTRIBUTION OF RED CELL DIAMETERS IN 14 NORMAL PERSONS, 7 MEN AND 7 WOMEN

Men ———, women - - - (From Ohno and Gievius 1925)

for women tend to run a little higher than those for men, the average for the former being 7.91 microns and for the latter 7.77 microns. The dispersion shows no appreciable difference for the two sexes.

The average of 0.91 micron for the dispersion of the whole group is somewhat less than ours and is undoubtedly due to the fact that they found fewer microcytes than we did. In general, however, their findings are in very close agreement with ours.

Our work in cases of pernicious anemia also confirms the findings of others. Increased dispersion (anisocytosis) probably occurs in all anemias, but we agree with Price-Jones that increased dispersion plus increased mean diameter is highly characteristic, in fact these two might be said to be the characteristics of the red cells in pernicious anemia as far as diameter is concerned. Anemias other than pernicious may resemble it in these respects, but pernicious anemia seldom if ever fails to show these phenomena. For this reason Hampson and Shackle (6) have suggested that from the histologic point of view it would be best to divide all anemias into megalocytic and non-megalocytic rather than into primary and secondary. In the megalocytic group they would include the anemias of sprue and diphtheriocephalus latus infestation.

Regarding the diagnostic significance of a distribution curve in differentiating between pernicious and other sorts of anemia, we agree with Hurst (1) that the finding of a normal or low mean diameter is very strong evidence against pernicious, or as he prefers to call it Addison's anemia. The average mean diameter in pernicious anemia found by Price-Jones (3) was 8.24 microns, by us 8.6 microns. The smallest and largest cell found by him were 3.75 microns and 12.25 microns respectively, and by us 4.0 microns and 13.9 microns.

It is of course desirable to learn the significance of the changes found in the blood of either pernicious anemia or other anemias. Why in pernicious anemia there should always be a high mean or median diameter, and in secondary anemia usually a normal or low one, is a question of great interest. Changes in mean diameter may result from physico-chemical changes in the blood, such as those produced by muscular work, forced breathing and alkali ingestion. From data of this sort Wiedmann and Schirmeyer (7) and Price-Jones (8) conclude that mean diameter is dependent on blood reaction, a shift to the acid side causing an increase in diameter, a shift to the alkaline side, a decrease. Our observations on the diameter of red

cells following strenuous muscular work agree with theirs<sup>1</sup>. It is probable however that physico-chemical factors alone are not capable of increasing dispersion significantly. Therefore, in the type of curve we use, rotation about the center in a clockwise direction denotes disturbance in blood formation or destruction, or both.

To interpret correctly the alterations found in any disease large numbers of data will have to be secured and correlations sought with a variety of other blood factors. Price-Jones (10) has already reported an absence of correlation between mean diameter and hemoglobin concentration of the blood or red count, but a definite correlation exists between both of these and dispersion in inverse ratio, that is to say, the severer the anemia, the greater the anisocytosis.

One thing that must be borne in mind is that whereas the red cell population of normal blood may follow a law of natural frequency, and therefore be a smooth curve, that of pernicious or other type of anemia may not. It may instead be a skew curve and represent a heterogeneous population. The study of the skewness of pathologic curves is therefore likely to yield information as to the nature of the changes occurring in blood disease. Difficulty will arise in distinguishing genuine from spurious skewness. In the counts of a relatively small number of cells the extremities of the curves denoting macrocytosis on the one hand and microcytosis on the other are based on such small numbers of observations that errors may be magnified, nevertheless with careful discrimination it should eventually be possible to recognize true skewness, or heterogeneity, and draw some conclusions on the probable significance of excess macrocytes or microcytes. Price-Jones (3) believes that in pernicious anemia there are probably three distinct populations first, abnormally large cells which he calls "the pernicious element," second, normal sized cells, and third, small cells due to the anemia resulting from extra blood destruction, "the anemia element." In many of our curves, as shown in figure 2, there is strong suggestion of genuine skewness indicating a heterogeneous population, perhaps of the sort Price Jones suggests.

<sup>1</sup> Since the completion of this paper we have seen the results of determinations of red cell diameter after strenuous exercise published by Dryerre, Millar and Ponder (9). They report no change in size.

The conclusions we have to draw at present are largely confirmatory of the work of others, they are as follows

#### CONC I USIONS

- 1 Diameters of the red cell population of the blood of normal persons follow the law of natural frequency
- 2 The medians and dispersions of such curves are remarkably alike among different subjects
- 3 In pernicious anemia there is a striking increase in both these functions
- 4 This fact is of diagnostic significance in distinguishing between pernicious and other types of anemia

We are greatly indebted to Prof E B Wilson for many invaluable suggestions

#### BIBLIOGRAPHY

- 1 Hurst, A G Brit Med Jour , 1924, i, 93 Addison's (Pernicious) Anaemia and Subacute Degeneration of the Spinal Cord
- 2 Price-Jones, C Brit Med Jour , 1910, ii, 1418 The Variation in the Sizes of Red Blood Cells
- 3 Price-Jones, C Jour Path and Bact , 1922, xxv, 487 The Diameter of Red Cells in Pernicious Anaemia and in Anemia Following Hemorrhage
- 4 Richardson, J G Am Jour Med Sci , 1877, ns, Ixxiii, 112 On the Identity of the Red Blood Corpuscles in Different Races of Mankind
- 5 Ohno, M, and Grievis, O Arch f d ges Physiol , 1925, cxx 315 Schwankungsbreite und Schwankungsart der Durchmesser menschlicher Erythrocyten
- 6 Hampson, A C, and Shackle, J W Guy's Hosp Rep , 1924, lxxiv, 193 Megalocytic and Non-megalocytic Anaemias
- 7 Wiechmann, E, and Schürmeyer, A Deutches Arch f klin Med , 1925, cxlvii, 362 Untersuchungen über den Durchmesser der roten Blutkörperchen
- 8 Price-Jones, C Jour Path and Bact , 1920, xxiii, 371 The Diurnal Variation in the Sizes of Red Blood Cells
- 9 Dryerre, H, Millar, W G, and Ponder, E Quart Jour Exper Physiol , 1926, xvi, 69 An Investigation into the Size of Human Erythrocytes Before and After Exercise
- 10 Price-Jones, C Guy's Hosp Rep , 1924, lxxiv, 10 Anisocytosis with Special Reference to Pernicious Anaemia

# THE EFFECT OF INCREASED HEART RATE DUE TO THE INJECTION OF ATROPINE ON THE OXYGEN SATURA- TION OF THE ARTERIAL AND VENOUS BLOOD OF PATIENTS WITH HEART DISEASE

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The present study was undertaken in an attempt to contribute to the solution of the problem of the effect of rapid heart rate on the oxygen saturation of the arterial blood. In 1921 Barcroft, Bock and Roughton (1) reported that they had found normal saturation of the arterial blood in a patient during an attack of paroxysmal tachycardia. Carter and Stewart (2) later reported a case in which there was a marked decrease in the oxygen saturation of the arterial blood during attacks of paroxysmal auricular tachycardia, and Dieuaide (3) had the same experience in a patient during attacks of paroxysmal ventricular tachycardia. Mealins (4) found normal saturation of the arterial blood with oxygen during regular and irregular tachycardia artificially induced in dogs. These dogs were under paraldehyde anesthesia and since the chests were open they were kept alive by artificial respiration. The author has had occasion to repeat these experiments under the same conditions and has confirmed these observations (5). More recently, Stewart, Crawford and Hastings (6) in their study of the effect of rapid heart rate on the blood flow in normal unanesthetized dogs found that the oxygen saturation of the arterial blood remained unchanged during rapid auricular fibrillation and was usually unchanged during regular tachycardia.

## METHODS

The following observations were made in cardiac patients who had been in the hospital at absolute rest for a long while and who at the time the observations were made showed no signs of decompensation. Patients having auricular fibrillation as well as those having normal rhythms were available, in one patient the

TABLE I  
The effect of increased heart rate on the oxygen saturation of the arterial and of the venous arm blood of patients with heart disease

Case number	Age	Rhythm	Time with reference to atropine injection	O <sub>2</sub> content				O <sub>2</sub> consumption	O <sub>2</sub> capacity arterial blood	Arterial blood	Venous blood	O <sub>2</sub> capacity of utilized blood	Diagnosis		
				mM	mM	mM	ccr						ccr	ccr	ccr
1	30	A T †	Before 16 min after	9 49 7 51 1	9 72	9 76	9 76	96	7 76	9 19 9	93	85	8	+†	Mitral insufficiency, chronic myocarditis
2	20	A. F.	Before 50 min after	9 66 5 60 4 0 6	9 76	9 59 1 38 9	—	75	63	7	—	—	—	—	Mitral stenosis and in- sufficiency, cardiac hypertrophy
		A. F. **	Before 24 min after	9 63 5 14 4 49	9 89	9 6 45 1 54 9	—	93	71	22	—	—	—	—	Mitral stenosis and in- sufficiency, cardiac hypertrophy
3	27	A. F.	Before 25 min after	8 17 5 19 2 98	8 45	95 6 60 9 34 7	—	77	77	0	—	—	—	—	Mitral stenosis and in- sufficiency, cardiac hypertrophy
4	20	A. F.	Before 20 min after	8 66 6 15 2 51	8 96	95 6 68 2 27 4	—	83	81	2	—	—	—	—	Mitral stenosis and in- sufficiency, aortic in- sufficiency, cardiac hypertrophy

5	38	A. F.	Before	9 54.5 58.3 96 9 91	95 3.55 9.39 4	86 70 16	+	Chronic myocarditis
		N. R.††	35 min after	9 39.5 53.3 86 9 89	94 0.55 5.38 5	0 125 76	49	
6	18	N. R.	Before	8 13.5 66.2 47 8 69	92 5.64 7.27 8	64		Chronic myocarditis
			24 min after	8 22.5 75.2 47 8 51	95 5.67 1.28 4	0	88	
7	20	N. R.	Before	9 61.7 42.2 19 10.06	94 6.73 4.21 2	80		Chronic myocarditis
			40 min. after	9 78.8 74.1 04 10.18	95 2.85 5.9 7	-	120	
8††	24	N. R.	Before	9 24.7 69.1 55 9 70	94 3.78 9.15 4	88	0†	Mitral stenosis and insufficiency; cardiac hypertrophy
			28 min. after	9 10.6 64.2 46 9 39	96 0.70 3.25 7	+	136	
			75 min after	3 61.1 64.1 97 3 79	92 9.42 2.50 7	84	0	Mitral insufficiency, car-
				3 62.1 56.2 06 3 89	90 7.39 1.51 6	0 118	75	dio hypertrophy; arterial hypertension, chronic nephritis, simple anemia

\* 0 —, and + In this column indicate no change, decrease and increase respectively

† A. F = auricular fibrillation.

† + indicates that the patient was under the influence of digitalis at the time the test was carried out, 0 indicates that the patient was not under the influence of digitalis at the time the test was carried out.

‡ The oxygen capacity of the venous as well as of the arterial blood was estimated in this test.

\*\* These observations were made one year after the first ones.

†† N. R. = normal rhythm. These observations were made 6 days after the ones made during auricular fibrillation

†† This patient was given 1.7 mgm. of atropine sulphate. The other patients were given 2 mgm.

observations were made during auricular fibrillation and later after the rhythm had returned to normal following the administration of quinidine sulphate. All the patients with auricular fibrillation as well as patients 5 and 6 (table 1) with normal rhythms were under the influence of digitalis at the time the observations were made. The plan was to study the oxygen saturation during a period of a slow cardiac rate and shortly afterward during a period of more rapid rate. The rapid rate resulted from the injection of atropine sulphate (2.0 mgm.) intravenously. The test was started at least 2 hours after the preceding meal. The patients lay quietly in bed for  $\frac{1}{2}$  hour before the test was begun. Several counts of the heart rate were then made at 5 minute intervals. A sample of arterial blood was taken from a radial or brachial artery and a sample of venous blood without stasis from a cubital vein. The patient continued to lie quietly while these blood samples were analyzed for their oxygen content. In the meantime several more control heart rates were taken. Atropine sulphate 2.0 milligrams was then given intravenously and after the maximum increase in heart rate had been present for a varying length of time second samples of arterial and of venous blood were taken. In drawing this sample of venous blood the needle was always inserted at the same point in the same vein from which the first sample had been obtained. Heart rates were counted at 5 minute intervals following the injection until the rate returned to normal, in order to be certain that the second blood samples were taken while the heart was still beating at the maximum rate. In patients with auricular fibrillation both the apex heart rate and the radial rate were counted and the pulse deficit plotted. The oxygen content of the blood samples was estimated by the Van Slyke and Neill manometric method (7). The oxygen capacity of the arterial blood was used in calculating the oxygen saturations.

#### OBSERVATIONS

There are six observations in 5 patients with auricular fibrillation and four in 4 patients with normal rhythm, in one of these (case 5) similar observations were made also during the presence of auricular fibrillation.

*The effect of the increased heart rate on the oxygen saturation of the arterial blood.* In no instance was there a conspicuous increase or decrease in the oxygen saturation of the arterial blood during the increased heart rate either in patients with normal rhythms or in those with auricular fibrillation (table 1). The heart rate rose as high as 187 per minute, the greatest increase in rate took place in the patients with auricular fibrillation. In one patient (case 2) similar results were observed in the two tests made approximately 1 year apart.

*The effect of the increased ventricular rate on the oxygen saturation of*

*the venous arm blood* In 3 patients with auricular fibrillation (cases 2, 3 and 4) (tables 1 and 2) and mitral stenosis the venous oxygen saturation was increased during the period of rapid rate, while in two patients without mitral stenosis but with auricular fibrillation (cases 1 and 5) it was unchanged One patient (case 7) with mitral stenosis and a normal rhythm showed a slight decrease in the venous oxygen saturation during the period of increased heart rate Of the other 3 patients having a normal rhythm one without valvular disease (case 5) and another with mitral insufficiency (case 8) showed no change in venous saturation during the time of increased heart rate, while in a third patient without valvular disease (case 6) venous saturation was increased during the period of faster rate (table 2)

TABLE 2

*The effect of increased heart rate on the oxygen saturation of the venous arm blood*

Number of cases	Rhythm and valve lesion	Venous saturation during increased heart rate
3	A. F *with mitral stenosis	Increased
2	A. F without mitral stenosis	Unchanged
1	N. R.† with mitral stenosis	Decreased
3	N. R. without mitral stenosis	Unchanged or increased

\* A. F = auricular fibrillation.

† N. R. = normal rhythm.

The results of these observations may then be summarized as follows

A In patients with heart disease, tachycardia *per se*, whether with regular or irregular rhythm, does not change the degree of *arterial* oxygen saturation

B During the time of rapid rate the degree of *venous* oxygen saturation is

- (a) *unchanged* in cases of valvular disease (other than mitral stenosis) irrespective of the rhythm
- (b) *increased* (1) in mitral stenosis if auricular fibrillation is present and
  - (2) with undamaged valves if normal rhythm is present
- (c) *decreased* in mitral stenosis if normal rhythm is present.

## DISCUSSION

In patients with heart disease, tachycardia whether regular or irregular did not decrease the arterial oxygen saturation *per se*. In one instance (case 2, 1925) the ventricular rate increased from 93 to 187 per minute, an increase of 100 per cent, in spite of this the oxygen saturation of the arterial blood remained unchanged (fig 1). The saturation of the venous arm blood increased from 52 to 81 per cent. How increased venous saturation occurred in this patient during tachycardia as well as in patients 3 and 4 is not easy to understand, since the number of effective beats during the period of rapid rate did not change. The point has been made by Goldschmidt and Light (8) that the oxygen content of the blood in the veins of the forearm is affected to some extent by variations in temperature and position, but these conditions remained unchanged in our observations.

The patient (case 5) in whom the test was first made when auricular fibrillation was present gave a similar response (fig 2) on repeating the test a few days later when the heart rhythm was normal. The venous saturation was higher during the normal rhythm as Stewart (9) has previously shown.

The increased venous saturation which occurs during increased ventricular rate in auricular fibrillation is not to be confused with the decreased saturation of the mixed venous blood which Stewart, Crawford and Hastings (6) have found to take place when the normally beating heart is made to fibrillate. In the latter case (decreased saturation) a change in rhythm has occurred. The increases in heart rate which followed the injection of atropine may have been within the limits to which the heart responds effectively by increased or unchanged minute volume output. Increases beyond these limits or increases in rate lasting for a longer time may be accompanied by failure on the part of the heart to maintain saturation. These increases in ventricular rate took place moreover without an increased demand on the heart by the organism.

These studies throw no light on the cause of arterial anoxemia in the cases reported by Carter and Stewart (2) and Dieuaide (3). Arterial saturation remains unaltered in the presence of extreme myocardial and valvular disease although the ventricular rate is very rapid,

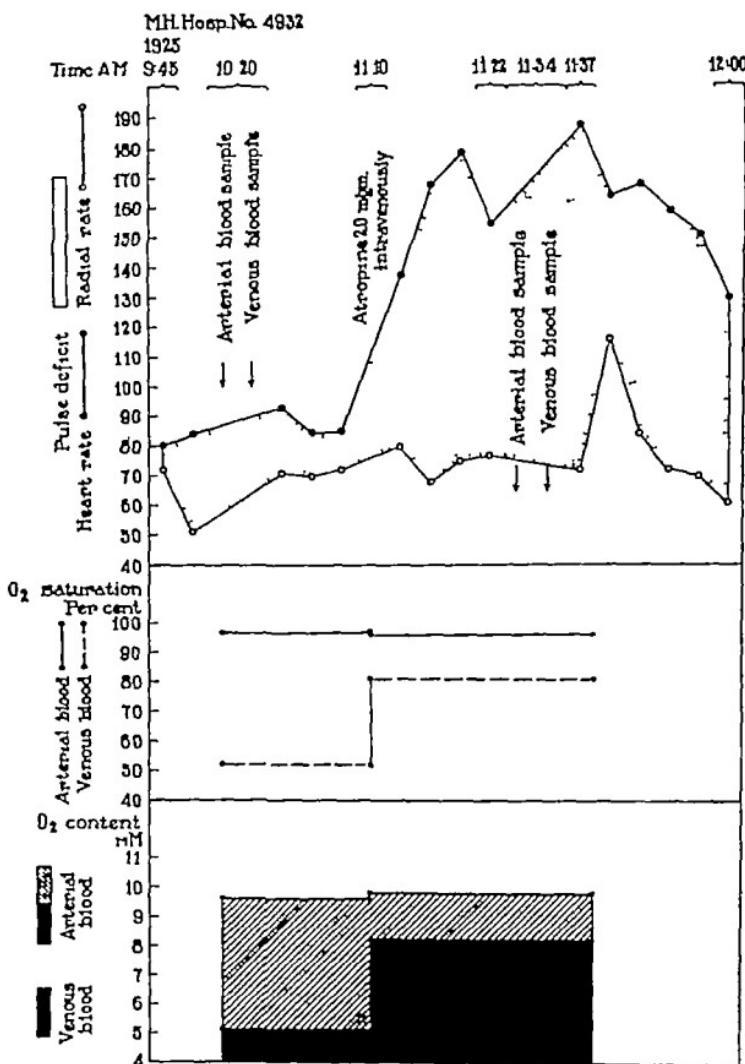


FIG. 1. This figure shows the effect of increased heart rate upon the oxygen saturation and the oxygen content of the arterial and of the venous arm blood in case 2 in 1925. The changes for convenience are represented as taking place immediately after the onset of the increased rate.

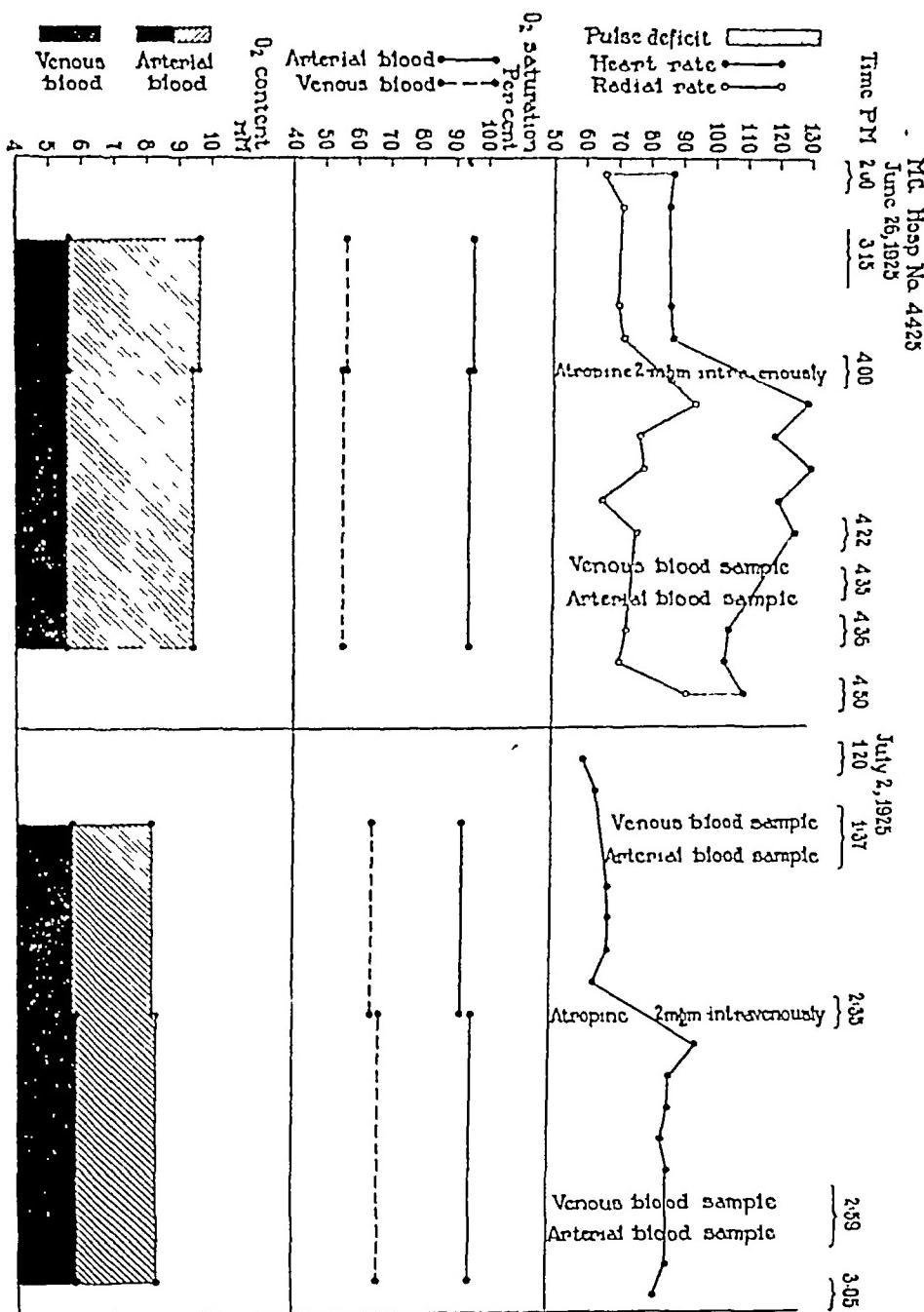


FIG. 2. This figure compares the effect of increased heart rate on the oxygen saturation and the oxygen content of the arterial and of the venous arm blood in case 5, during auricular fibrillation and later during the normal rhythm. For convenience the changes are represented as taking place immediately after the onset of the increased heart rate.

as these and other observations show (case 2, table 1) In the cases mentioned above there may have been pulmonary congestion which interfered with complete oxygenation of the blood in the lungs There were however no râles over the chest in either of these cases On the other hand sufficient congestion to interfere with normal oxygenation of the blood may have been present without giving rise to enough moisture to produce râles The myocardial damage may have been too great for the heart to maintain an adequate circulation during paroxysms of tachycardia, the oxygen unsaturation of the venous blood becoming so marked that it could not be raised to the normal level of arterial blood in the pulmonary circulation time Once the venous blood has become unduly unsaturated an additional burden is placed on the heart, because of increased viscosity of unsaturated blood (10) The increased viscosity of unsaturated blood may play an important and hitherto unstressed rôle in the onset and continuance of heart failure especially when the large size of the vascular bed is considered

In patients with mitral stenosis, the difference in behavior during irregular tachycardia and in the presence of the normal rhythm corresponds with the clinical impression that they often seem better after the onset of auricular fibrillation than when the rhythm was normal On studying from this point of view the data which the author (9) published on oxygen saturation of the arterial and venous arm blood in auricular fibrillation and after restoration of the normal rhythm, it is found that there were only two patients (cases 4 and 9), in whom the venous oxygen saturation did not increase following the return to the normal rhythm, both of these patients had mitral stenosis One of these patients was subjectively improved following the return to the normal rhythm, while the other one was miserable until fibrillation returned. In the other 7 patients without mitral stenosis the venous saturation increased.

If increased venous saturation may be taken as a measure of improvement in the circulation these observations may be interpreted in the following manner In mitral stenosis, it is after the onset of auricular fibrillation that improvement takes place as an accompaniment of increased venous saturation In the absence of mitral stenosis, the circulation is not improved by the onset of auricular

fibrillation, increased venous saturation does not occur. The determining factor then is mitral stenosis, in its presence, auricular fibrillation is an advantage, without it, a disadvantage. This correlation is however too curious to permit its being maintained on the basis of these few observations. Undoubtedly the state of the heart muscle as well as the dynamics of the blood flow must be taken into account before the adoption of definitive opinions.

#### CONCLUSIONS

1 Tachycardia *per se* whether regular or irregular does not affect the oxygen saturation of the arterial blood in patients with heart disease.

2 In this series of observations it appears that tachycardia may be followed by increased oxygen saturation of the venous arm blood in patients without mitral stenosis in the presence of a normal rhythm and in patients with mitral stenosis in the presence of auricular fibrillation.

#### BIBLIOGRAPHY

- 1 Barcroft, J., Bock, A. V., and Roughton, F. J. Heart, 1921-22, ix, 7 Observations on the Circulation and Respiration in a Case of Paroxysmal Tachycardia
- 2 Carter, E. P., and Stewart, H. J. Arch. Int. Med., 1923, xxxi, 390 Studies of the Blood Gases in a Case of Paroxysmal Tachycardia
- 3 Dieuaide, F. R. Johns Hopkins Bull., 1924, xxxv, 229 Observations on the Respiratory Gases in Ventricular Paroxysmal Tachycardia
- 4 Meakins, J. Heart, 1921-22, ix, 185 The Influence of Circulatory Disturbances on the Gaseous Exchange of the Blood I The Oxygen Saturation of the Arterial Blood in Tachycardia
- 5 Experiments to be published
- 6 Stewart, H. J., Crawford, J. H., and Hastings, A. B. Jour. Clin. Invest., 1926, iii The Effect of Tachycardia on the Blood Flow in Dogs I The Effect of Rapid Irregular Rhythms as Seen in Auricular Fibrillation Stewart, H. J., and Crawford, J. H. Jour. Clin. Invest., 1926, iii, The Effect of Tachycardia on the Blood Flow in Dogs II The Effect of Regular Rapid Rhythm
- 7 Van Slyke, D. D., and Neill, J. M. Jour. Biol. Chem., 1924, lxi, 523 The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement I

- 8 Goldschmidt, S , and Light, A. B Amer Jour Physiol , 1925, lxxii, 146  
The Effect of Local Temperature upon the Peripheral Circulation and Metabolism of Tissues as Revealed by the Gaseous Content of the Venous Blood
- 9 Goldschmidt, S , and Light, A. B Amer Jour Physiol., 1925, lxiu, 127  
A Comparison of the Gaseous Content of the Blood from Veins of the Forearm and the Dorsal Surface of the Hand as Indicative of Blood Flow and Metabolic Differences in These Parts
- 10 Stewart, H. J Arch. Int. Med , 1923, xxxi, 871 Observations on the Blood Gases in Auricular Fibrillation and After the Restoration of the Normal Mechanism.
- 11 Odaira, T Tohoku Jour Exp Med., 1921, iv, 396 The Influence of the Oxygen Content of Blood upon Its Viscosity



# INFLUENCE OF PREVIOUS SALT REGIME ON EXCRETIONS OF CHLORINE, SODIUM, AND POTASSIUM DURING THE CHLORIDE CONCENTRATION TEST OF DE WESSELOW

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## I CHLORIDE EXCRETION

There have been many attempts to estimate renal function from a study of urinary chloride excretion or concentration. The salt test introduced by Schlayer (1) was later discredited because of the effect of extra renal factors. It was shown particularly by Rowntree and Fitz (2) that a previous salt free diet placed the organism in a state of apparent salt unsaturation, and that a subsequently administered dose of salt would be retained unusually long, even though the kidneys were normal. However, de Wesselow (3) has recently proposed a somewhat different test for renal chloride excretion. He gives 4 grams of KCl in 200 cc. of water without regard to the previous diet or regime of the patient, and determines the chloride concentration of the urine during each of the three subsequent hours. The maximum chloride concentration (normally approximating 0.90 per cent Cl) is taken as indicating the maximum concentrating power of the kidney.

De Wesselow's test differs from Schlayer's in that he uses KCl instead of NaCl, and bases his conclusions upon the maximum chloride concentration attained in the urine rather than upon the period of time required for complete elimination of the ingested dose.

It seemed desirable to ascertain whether the de Wesselow test would yield results reasonably independent of the previous regime of the patient. We have, therefore, tried it on three patients after alternating chloride-free and chloride-containing diets.

### *Method of Investigation*

The patients were selected to give a considerable variation in age, general health, type of nephritis and state of renal function. None

had subcutaneous edema or fluid in the serous cavities at the time the investigation was begun. On each type of diet sufficient time was allowed for a balance to be struck between salt intake and output or for the weight to become constant. In case 1, however, salt feeding had to be interrupted sooner than planned, owing to the onset of nausea and vomiting. The de Wesselow test was carried out at the end of each particular diet-period, the salt intake was then changed, and the test was repeated after an equilibrium between salt ingested and salt excreted had been again attained. Not over 6 grams of salt a day was added to the diet during the period of salt administration. The salt-free diet averaged less than one gram of salt a day, estimated from the known salt content of the milk in the diet, the other foods being so chosen as to contain only insignificant quantities of salt, which are not included in the estimate of the daily salt intake.

The test itself was begun from 45 minutes to 90 minutes after breakfast. Four grams of KCl were given with 200 cc of water by mouth and the urine was collected hourly for three consecutive hours, during which period no food nor fluid was allowed. The urinary chloride concentration was determined by the usual Volhard titration method. The urea concentration index, indicating the number of times the kidneys concentrate the blood urea in the urine, was determined as described in a previous paper from this laboratory (4).

#### *Case reports*

*Case 1* G. O., age 48, carpenter, was admitted November 4, 1925, complaining of shortness of breath. Nocturia, polyuria and dyspnea on exertion came on during the preceding summer, but in the three weeks prior to admission the dyspnea had become very severe, preventing sleep. Associated symptoms were cough, oliguria, occipital headache and impairment of vision. There was no history of hematuria nor of edema. There had been scarlet fever in childhood. Examination showed a patient in severe dyspnea, with some cyanosis, signs of pulmonary congestion, marked generalized arteriosclerosis, B. P. 230/164, the left heart border 12 cm from the midsternal line in the fifth interspace, gillot rhythm, a rough systolic aortic murmur, some enlargement of the liver, no ascites, slight edema of the legs and over the sacrum, arteriosclerosis of the retinal vessels and hemorrhages in the fundi. The urine showed albumin, few erythrocytes, many hyaline and granular casts. Digitalization produced marked clinical improvement within a week, and the edema disappeared. The hypertension and headaches continued and the eye-ground changes advanced to those of a typical

albuminuric retinitis. The functional findings indicate an advanced state of renal insufficiency. The patient's condition was fairly stable during the period of investigation.

*Case 2* R. V., age 24, streetcar conductor, was admitted November 17, 1925, complaining of persistent edema of the legs. He never had a sore throat, but frequent colds in the head. After a rather severe cold in June, 1925, marked edema set in, along with nocturia, weakness, and shortness of breath upon exertion. Under proper treatment the edema cleared up somewhat and marked loss of weight was evident, due to loss of body tissue. Infected tonsils were removed in July, 1925. There was some improvement, but weakness, pallor, and edema of the legs hung on. Examination showed moderate pallor, no evidence of oral, nasal, or paranasal sinus infection, the left heart border 10.0 cm. from the mid sternal line in the 5th interspace, a slight systolic murmur, B.P. 148/76 no fluid in the chest or abdomen, slight edema of the legs, the urine loaded with albumin, erythrocytes, leucocytes, fatty cells, and hyaline, granular and cellular casts, the eye grounds negative. The edema cleared up rapidly but all the other findings remained obstinately stationary. Renal function in this case is only moderately impaired as compared with the state of case 1.

*Case 3* R. G., age 11, school boy, was admitted December 22, 1925, complaining of edema of the face and legs. This began four weeks previously, after an attack of acute abdominal pain, nausea and vomiting, without gross hematuria. There was no preceding acute infection but the patient had a chronic discharging mastoid sinus dating back to the original operation in January, 1924. There was no history of scarlet fever or sore throats. Examination showed a patient with mild bronchitis, puffiness and pallor of the face, a small draining sinus over the left mastoid region with local inflammatory edema of the scalp, considerable adenopathy of the left cervical chain, the left heart border 8.5 cm. from the midsternal line in the fifth interspace, a slight systolic blow at apex and base, B.P. 120/82, a small amount of fluid in the right chest, no ascites, moderate edema of the legs and over the sacrum, a leucocyte count of 17,600 normal hemoglobin, and in the urine much albumin, many erythrocytes few leucocytes, and hyaline, granular and a few epithelial and red blood cell casts. The functional findings were nearly normal. Edema subsided rapidly and the general condition was good until January 19, 1926 when the mastoid sinus stopped draining. Fever, edema, and oliguria set in. On January 21 the walls of the sinus were incised to give better drainage. This was followed two days later by a sharp pyrexia, abdominal pain, nausea, vomiting, lumbar tenderness increased hematuria, edema and leucocytosis. Diuresis and loss of edema followed in two days. A hemolytic streptococcus was grown from the pus in the mastoid sinus. In this case as contrasted with the preceding two, renal function is now essentially normal, although temporary variations have occurred. In fact the urea concentration index has been persistently just at, or a little below, the lower limit of the normal range, in the

presence of an excellent phthalein output and a low blood urea nitrogen. During the last period of observation, noted in table 1, the urea concentration index indicates perfectly normal renal function for the first time.

### *Discussion of Results*

Table 1 gives a summary of the chief functional findings in the three cases studied and illustrates the variations produced by the preceding type of diet—salt-containing or salt-free—in the de Wesselow chloride concentration test. The figures for the urea concentration index and the phthalein output represent the average of the determinations during the particular period. The maximum urinary chloride concentration was usually found in the third hour after administration of 4 grams of potassium chloride with 200 cc water. Sometimes, however, this occurred in the second hour and was the figure used. Insufficient urinary volume was not a factor in this series of cases.

The most striking fact apparent from the table is the variation in the maximum urinary chloride concentration shown by each patient. In each of the three cases the de Wesselow test gave results indicating, apparently, much better chloride concentrating ability when the patient was on a salt-containing diet than when he was on a relatively salt-free diet. The difference between the values for the two periods amounted to several hundred per cent at times. That this difference in chloride concentration did not reflect a corresponding change in renal function is proved by the relatively small variation in the urea concentration index and phthalein figures. The consistency with which the chloride concentration went back to a low level when the patient was returned to a salt-free diet—other things remaining essentially equal—points to an extra-renal factor. This factor seems to be the chloride want of the body as a whole. When a patient has been on a salt-free diet for some time the tissues are depleted of their reserve chloride content, although the blood plasma may maintain a normal chloride concentration. Salt ingested under such conditions probably passes rapidly out of the circulation and into the tissues, becoming unavailable to the kidneys. When, on the contrary, as a result of several days of salt feeding, the tissues have saturated themselves with chloride, a test dose of salt is made rapidly available to the kidneys, and excretion, with concentration, follows.

TABLE 1  
*The effect of salt in the diet upon the urinary Cl concentration in the de Wesselow test*

Case number	Name	Age	Period of observation	Daily NaCl intake	Urea concentration index $\frac{U}{B} \sqrt{\frac{V}{W}}$	Phtha- lein in 2 hours	Maximum urinary Cl concentration in de Wesselow test	
							per cent	per cent Cl per liter
<i>years</i>								
1	G. O.	48	<i>1925</i>					
			January 23 to February 4	2.0	7	6	0.12	34
			February 5					
			February 8 to February 15	5.4	6	6	0.28	79
			February 16					
			February 17 to February 26	0.1	6	6	0.07	20
			February 27					
2	R. V.	24	<i>1925-1926</i>					
			December 5 to January 20	0.5	21	43	0.13	37
			January 21					
			January 23 to January 31	5.0	21	34	0.46	130
			February 1					
			February 4 to February 12	0.5	—	32	0.19	54
			February 13					
3	R. G.	11	<i>1925-1926</i>					
			December 23 to February 3	0.4	31	71	0.32	90
			February 4					
			February 5 to February 15	5.2	—	80	0.54	152
			February 16					
			February 18 to February 26	0.5	37	71	0.11	31
			February 27					
			February 28 to March 10	0.5	32	76	0.27	76
			March 11					
			March 12 to March 17	5.7	36	65	0.57	161
			March 18					
			March 21 to March 31	0.5	48	59	0.23	65
			April 1					

\* The Index is normally always above 35 U = urea concentration in urine B = urea concentration in blood, V = cc. urine excreted per hour, W = body weight in kilos. The index has been discussed in a previous paper from this laboratory (4). It indicates the number of times the kidneys concentrate the blood urea in the urine, when the volume output is 1 cc. per kilo per hour.

† The maximum in normal subjects was found by de Wesselow to be 0.8 to 1.0 per cent of Cl, equivalent to 220 to 280 mM per liter.

The use of sodium or potassium chloride perorally as a means of determining the concentrating ability of the kidney becomes practical, therefore, only when extra-renal factors are controlled. In view of the many complex inter-relationships between the chloride ion and other electrolytes in the maintenance of the water balance between body-fluids and cells, in the regulation of osmotic equilibrium, and in the acid-base mechanism, it would appear difficult to make any chloride test consistent. The situation is much more simple in the case of urea, because of its uniform distribution throughout the body as a whole and its relative unimportance in the equilibria mentioned above. Perhaps, if one could load the body sufficiently with chloride—by diets containing much salt—extra-renal factors would be minimized for a chloride test. Such a procedure, however, would be dangerous in many instances.

## II SODIUM AND POTASSIUM EXCRETION

The eliminations of sodium and potassium during the de Wesselow test were studied in order to determine which base accompanies the increased chlorine output. Studies of mineral metabolism in infants by Schloss (7) and by Meyer and Cohn (8) have shown that administration of KCl in relatively large doses (2 grams) causes a diuresis with an accelerated output of sodium as well as potassium. That a similar result follows in adults with varying types of nephritis could not, however, be concluded without direct evidence. Because the time available for this portion of the work was limited, experiments were completed on only two patients, and the periods of observation were limited to the three hours following the administration of KCl, as in the de Wesselow test. The aim has been limited to the answer of the question Does the increased chloride output following the administration of KCl in the de Wesselow test indicate merely excretion of the administered potassium chloride, or is there also a washing out of sodium?

As in the experiments on chloride excretion, the patients were alternated on salt-free and salt-containing diet periods. Each regime was continued until the patient was in chloride equilibrium, or until the body weight had become stationary.

A control test was then carried out in which the patient received 200 cc. of water at the beginning of the test, and voided every hour thereafter for three consecutive hours. This was done as a control on the possible diuretic action of water itself. On the following day, a regular de Wesselow test was carried out, the patient receiving 4 grams of KCl and 200 cc. of water. Cases 2 and 3 were studied in this manner.

#### *Methods of Analysis*

The hourly specimens of urine were analyzed for chloride either by the usual Volhard titration or, where only small amounts of urine were available, by the plasma chloride method of Van Slyke (5).

Sodium and potassium were determined by the method of Goto (6) slightly modified as follows:

To 20 to 40 cc. of urine in a 50 cc. volumetric flask, 10 cc. of 25 per cent trichloroacetic acid were added to precipitate the protein, which was regularly present in the urines of these patients. The flasks were filled up to the marks with water and the solution was passed through an 11 cm. ash free dry filter paper. Of the filtrate 40 cc. were placed in a silica dish with 0.5 cc. of concentrated sulfuric acid, and evaporated as far as possible on a water bath. The dish was then transferred to an electric hot plate and heated, the temperature being raised slowly to avoid spattering. After evolution of fumes had nearly ceased, a few more drops of concentrated sulfuric acid were added to the charred mass, and the heating was repeated until fumes ceased coming off. The dish was then heated over a triple Bunsen burner for 30 minutes to burn off the carbon. The residue, which frequently contained a little carbon, was dissolved in 15 cc. of water and 5 cc. of concentrated hydrochloric acid. The solution was transferred to a 150 cc. flask, and the silica dish was washed into the flask with 4 portions of 5 cc. each of 0.5 N hydrochloric acid, which was warmed each time in the dish. To remove phosphoric and sulfuric acid from the solution in the flask, 3 cc. of 10 per cent barium chloride were added, and a 10 per cent suspension of calcium hydroxide in sufficient amount to turn the mixture just alkaline to litmus. Usually 25 to 30 cc. of the lime suspension were required. The mixture was diluted up to volume and filtered through an ash free 11 cm. folded paper. To remove barium and calcium from the filtrate, 100 cc. of the latter were treated in a 200 cc. flask with 25 cc. of a saturated ammonium oxalate solution and then ten per cent ammonium carbonate solution was added until no further precipitation occurred and the supernatant solution became clear. Usually 30 to 40 cc. of ammonium carbonate solution were required. The mixture in the flask was filled up to the 200 cc. mark with water, and filtered. Of the filtrate 50 cc. portions were placed in weighed silica dishes, and 5 cc. of concentrated hydrochloric acid were added to

each. The solutions were evaporated to dryness on the water bath, and were then heated on an electric hot plate until no more fumes of ammonium chloride were given off. Each dish was then heated cautiously over a small free flame just to a temperature sufficient to melt the mixture of alkali chlorides. (Over-heating would cause loss by volatilization.) The combined weight of the KCl and NaCl was determined by weighing the cooled dishes.

The potassium was determined as perchlorate. To the combined chlorides in the silica dish 3 or 4 cc of water were added, and 5 drops of perchloric acid of 1.12 specific gravity. The mixture was evaporated to dryness, and the residue was stirred up with 97 per cent alcohol containing 0.2 per cent of perchloric acid. The mixture was permitted to stand 20 minutes or more, and was then transferred to a weighed Gooch crucible. For completion of the transfer and washing 15 to 20 cc of the same alcoholic solution were used. The precipitate of  $KClO_4$  was dried for an hour or more at 110° and weighed. The KCl calculated from the  $KClO_4$  found was subtracted from the combined weights of KCl and NaCl previously found, and the NaCl thus found by difference.

Blank analyses were done on the reagents, which proved to be free of potassium, but yielded 6.0 mgm of NaCl. This was subtracted from the weight of the combined chlorides obtained in the urine analyses.

The precipitates weighed were often under 20 mgm, because of the necessity of using rather limited samples of urine collected during the hourly periods, and because of the small salt content of the urines, especially those collected during the periods in which the subjects were on approximate-chloride-free diets. Control analyses with similar amounts of pure KCl and NaCl gave quite good results, and we are confident of the approximate accuracy of the urine determinations. That the urines contained traces of sodium during the periods in which the analyses give negative results for this alkali, however, is probable, for the amounts analysed were not sufficient to show traces of the substance.

In a control analysis a standard solution was made up containing 1.0035 grams of NaCl and 1.0059 grams of KCl in 250 cc of water, 20 cc of this solution was carried through the above procedure. Duplicate 50 cc portions of the final filtrate yielded 20.5 and 21.4 mgm of NaCl + KCl, after subtracting the blank of 6.0 mgm. The theoretical value was 21.4 mgm. The weights of  $KClO_4$  were 19.1 and 19.7 mgm, equivalent to 10.3 and 10.6 mgm of KCl. The theoretical value was 10.7 mgm. By subtraction, NaCl was present to the extent of 10.2 and 10.8 mgm, respectively, theoretical yield being 10.7 mgm.

## DISCUSSION OF THE BASE EXCRETION

The two cases were quite different in their tendency to form edema, and they showed accordingly different reactions to the ingestion of potassium chloride in the de Wesselow test (see table 2)

R. V was a young man in whom an acute nephritis beginning about a year before had developed into a subacute chronic nephritis, with urea concentrating and phthalein excreting power reduced to about half normal, and with only a moderate tendency to form edema. When 5 grams of salt were added to his diet he would retain water up to a certain amount and show some weight increase, but in a few days, would reach a state of salt equilibrium, with output equal to intake and weight stationary, and without massive edema formation. When he was returned to a salt-free diet he readily eliminated the fluid previously retained.

R. G on the other hand was a boy who would readily have been classed as a pure nephrosis case were it not for the constancy of hematuria, and a history of acute nephritis. His renal function was normal for urea and phthalein, but he had a tendency to edema formation difficult to control. During months of stay in the hospital on a salt-free diet he was never free from visible edema, which increased markedly when 5 grams of salt were added to the diet, and returned to its former state but slowly when the salt was discontinued.

When both patients had been on a salt-free diet for a sufficient period to reduce the saline stores of their bodies to the minimum thereby attainable, both reacted to the KCl administration with increased potassium output, accompanied by decrease rather than increase in the hourly Na output.

When, however, both had been on a diet with 5 to 6 grams of NaCl in it, they did not behave alike under the de Wesselow test. In the man R. V there was an actual decrease in potassium output in the first and second hours, but an increased sodium output (unfortunately the urine of the third hour was lost). In the relatively much more edematous boy, R. G., there was increase in the potassium output, which did not become definite until the second hour, and it was accompanied by a decrease in sodium output.

TABLE 2  
*The excretion of Cl, K and Na in the urine during the de II test or and II c control tests, on salt-free and salt-containing diets*

Case number	Name	Date	Daily NaCl intake	Solution given at beginning of test	Cl		K per hour	Na per hour	$\text{Na} + \text{K}$ per hour	Urine volume per hour	
					per cent	mm. Fr. per hour					
2 R V		1926	200 cc H <sub>2</sub> O		0.016	17.0	0.48	103.0	2.64	0.0	
		June 7	0.9		0.055	52.2	1.47	129.0	3.31	0.28	
				4 grams KCl + 200 cc H <sub>2</sub> O	0.046	43.7	1.23	107.2	2.75	17.5	
		June 8	0.9		0.058	37.0	1.04	127.5	3.27	24.5	
June 15		1926	200 cc H <sub>2</sub> O		0.109	89.3	2.52	188.3	4.82	0.0	
					0.158	119.0	3.35	156.0	4.00	3.3	
				4 grams KCl + 200 cc H <sub>2</sub> O	0.301	186.7	5.26	161.6	1.14	46.1	
					0.276	248.5	7.00	210.2	6.16	93.6	
June 16		1926	200 cc H <sub>2</sub> O		0.317	221.9	6.24	151.6	3.88	65.2	
				4 grams KCl + 200 cc H <sub>2</sub> O	0.243	112.0	3.15	104.7	2.68	76.4	
					0.297	256.0	7.21	213.5	5.47	133.7	
					—	—	—	—	—	—	
3 R G		1926	200 cc H <sub>2</sub> O		0.044	25.7	0.72	76.2	1.95	59.1	2.57
		June 15	0.6		0.111	33.1	0.94	68.1	1.75	21.5	0.93
					0.059	44.7	1.26	116.3	2.98	46.4	2.02
June 16		1926	4 grams KCl + 200 cc H <sub>2</sub> O		0.026	30.0	0.84	131.0	3.36	13.3	1.88
					0.089	57.6	1.62	204.0	5.23	29.1	1.26
					0.151	60.4	1.70	197.0	5.05	3.5	0.15



We do not wish from these two experiments to generalize on the relationship of "tendency to edema" and the efficiency of potassium salts in stimulating excretion of the sodium-containing edema fluids. But clinical experience with patients other than those tabulated here has shown that in some cases with great tendency to dropsy the addition of 10 grams of KCl daily to a previously chloride-free diet results in fluid retention instead of loss.

In both cases presented in table 2, the KCl administration during the periods with 5 to 6 grams of salt in the daily diet had but little effect on the Cl concentration in the urine. The chloride concentrating ability of these patients could have been determined as well without the administration of the potassium chloride as with it.

#### SUMMARY

The urinary chloride concentration test of de Wesselow was studied under controlled conditions upon three patients differing in age, general health, type of nephritis, and state of renal function.

The maximum chloride concentration obtained when the patients were consuming 5 to 6 grams of salt per day varied from 0.28 per cent Cl in case 1 to 0.57 per cent in case 3. With a salt-free diet the values ranged from 0.07 per cent Cl in case 1 to 0.32 per cent in case 3. Urea concentration index and phthalein were unaffected by the changes in salt intake. The results of the de Wesselow test, even when not complicated by the extrarenal factors present in edema, evidently depend greatly upon the salt content of the diet during the days preceding the test.

Comparison of results obtained with the different patients after diets of similar salt content shows a rough tendency for the chloride concentrating power, indicated by the de Wesselow test, to follow the urea concentrating power and phthalein output, but no approach to exact parallelism.

The hourly excretion of potassium and sodium during the de Wesselow test were followed in two nephritic patients. One (R.G.) had a maximal dropsical tendency, the other (R.V.) a moderate one. During the salt-free dietary periods, administration of KCl in the de Wesselow test decreased the sodium output in both patients. During

the salt containing dietary periods the de Wesselow test caused the more edematous patient to retain sodium and the less edematous one to lose it

In both patients the Cl concentration of the urine depended much more on the daily intake of NaCl than on the KCl given in connection with the test

#### BIBLIOGRAPHY

- 1a Schlayer and Takayasu, R. Deut Arch, klin Med., 1910, xcvi, 17 Untersuchungen über die Funktion kranker Nieren
- 1b Schlayer and Takayasu, R. Deut Arch klin, Med., 1911, ci, 333 Untersuchungen über die Funktion Nieren beim Menschen
- 1c Schlayer Deut. Arch klin Med., 1911, cu, 311 Untersuchungen über die Funktion kranker Nieren bei chronische vaskulare Nephritiden
- 2 Rowntree, L G, and Fitz, R. Arch Int. Med. 1913, xi, 258 Studies of Renal Function in Renal, Cardiorenal and Cardiac Diseases.
- 3 deWesselow, O L V Quart. J Med., 1925 xix, 53 The Excretion of Chlorides by the Healthy and Diseased Kidney
- 4 Van Slyke, D D, Linder, G C, Hiller, A., Leiter, L, and McIntosh, J F J Clin, Inv., 1926, ii, 255 The Excretion of Ammonia and Titratable Acid in Nephritis
- 5 Van Slyke, D D Jour Biol Chem., 1923, lviii, 523 The Determination of Chlorides in Blood and Tissues.
- 6 Goto, K. Jour Biol Chem., 1918, xxxvi, 355 Mineral Metabolism in Experimental Acidosis.
- 7 Schloss, E. Zeit, Kinderheilkunde, 1912, ii, 441 Die Wirkung der Salze auf den Säuglingsorganismus auf Grund früherer und neuerlicher Untersuchungen
- 8 Meyer, L F, and Cohn, S. Zeit Kinderheilkunde, 1911, ii, 360 Klinische Beobachtungen und Stoffwechselversuche über die Wirkung verschiedener Salze beim Säugling



## THE SURFACE TENSION OF THE BLOOD SERUM IN NEPHRITIS

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This study had as its aim the collection of data on the surface tension of the serum in the various types of renal disease, and the correlation of these with other manifestations of the disease. The problem of edema was the focus of interest. On the one hand, low surface tension has been connected etiologically with edema by Clausen (1), who isolated from the urine a surface active substance capable of altering the permeability of collodion membranes to protein solutions. It appeared that at least one factor in edema formation might be altered permeability of the capillaries due to this surface active substance. On the other hand the clear-cut work of Govaerts (2) shows a constant relationship between nephritic edema and a lowered osmotic pressure of the plasma colloids, due to the low albumin content. We have, therefore, studied nephritic edema from the point of view of its relation to surface tension on the one hand, and to the plasma proteins, more particularly the plasma albumin, on the other.

There is in the literature a fair amount of data concerning surface tension in health and disease, most of the values having been obtained by means of the Traube stalagmometer. Kisch and Remertz (3) observed the relatively low values in the case of cloudy or milky sera and those colored by hemoglobin. They found that the cerebrospinal fluid has practically the same surface tension as distilled water, and ascribed this to the low concentration of colloids in it. Traube (4) included a series of nephritic cases in his tables on surface tension of the blood serum in various diseases. It is interesting, in view of the work of Clausen (5), to note that Traube had low values for some of the nephritic sera which were also cloudy or milky. Clausen (5) has recently published data on a considerable series of normal subjects and patients with nephritis. He found a distinct lowering of surface

tension, as determined by the drop-weight method, in those patients with so-called parenchymatous nephritis or with nephrosis. There was some correlation between the low surface tension, the low plasma protein level, the high degree of albuminuria, and the presence of edema.

In the present study are included patients with different types of nephritis. The surface tension was determined on undiluted serum and on serum diluted 100 times with 0.9 per cent NaCl solution. In addition, the time curve of the fall in surface tension that occurs in serum standing quietly, described by duNouy (6), was followed in about half of the cases studied.

#### METHOD OR PROCEDURE

The instrument used for the determinations was the original, indirect-reading tensiometer of duNouy (7). The time-change measurements were made with the aid of the special table and the carriage on rollers for the tensiometer (8), kindly loaned to us by Dr. duNouy. The watch-glasses were 5 cm in diameter and of standard curvature and depth. Two cubic centimeters of fluid were used. All the glassware, except the syringes for drawing blood, was boiled in potassium dichromate-sulphuric acid cleaning mixture, rinsed in distilled water and allowed to dry. The watch-glasses were not touched by the hands and were always flamed prior to using.

Blood was usually drawn from one to three hours after breakfast. It was allowed to clot and was centrifuged to separate the serum, which was pipetted off and kept at room temperature until used. Dilutions of serum were made with 0.9 per cent sodium chloride. The actual measurements were always begun within 4 or 5 hours from the time the blood was drawn. They were done at a room temperature of from 21° to 24°C. Except in the case of undiluted sera, all but a few of the determinations were repeated one or more times. The readings usually agreed within one per cent. The value obtained for distilled water at 23°C was 78.2 dynes per centimeter.

#### DISCUSSION OF RESULTS

The material studied included seven cases of active or convalescent acute nephritis, nine cases of chronic nephritis with varying degrees

of impairment of renal function, three cases of chronic lipid nephrosis, and one case which began apparently as nephrosis but later developed typical findings of chronic nephritis with renal insufficiency. All but one of these patients (case 16) had edema to a varying extent at one time or another during the course of the disease. The tables indicate the state of edema during the period when the surface tension measurements were made. The absence of edema may mean either that the patient was free from any tendency to edema or, more frequently, that the salt-free diet and rest in bed prevented the development of edema. The plasma protein figures were for the most part obtained from blood drawn a number of days before or after the surface tension determinations with which they are listed in the tables. However, the relative constancy of the plasma protein level in these patients justifies the use of the data for comparative purposes. The blood pressure figures represent averages of several determinations taken a few days before and after the surface tension measurements. The urea concentration index figures were obtained on the dates when they are listed or within a day or two of these dates. The manner of calculating the index has been described in a previous paper from this laboratory (9). The gross appearance of the serum has been characterized by the terms "clear," "slightly opalescent," "opalescent," "milky" and "creamy," in the order of increasing turbidity.

Most of the surface tension values were obtained on serum diluted 100 times with 0.9 per cent sodium chloride solution. It was found that such figures varied in the same direction as those of undiluted serum. Furthermore, instead of requiring 4 cc of serum for duplicate determinations, 0.5 cc. was sufficient. Finally, the actual readings could be carried out more simply and accurately upon the diluted than upon the whole serum, because the surface tension does not fall so rapidly in the case of the former. The values for serum diluted a hundred fold averaged 6.1 dynes higher than those for whole serum, the minimum difference being 4.5 dynes and the maximum 8.8 dynes.

*Acute nephritis.* In this series of seven cases, only case 2 still had marked impairment of renal function when the surface tension was determined. The rest were either convalescing or showing a tendency to go into a mild chronic state with little or no impairment of renal function.

TABLE 1  
The surface tension of the serum in acute nephritis

Case number	Name	Age	Date	Appearance of serum	Surface tension of serum in mm per centimeter	Diluted 1/100		Room temperature		Plasma proteins		Blood pressure		Remarks
						Diluted	Total	Albumin	Albumin	Urea	Urea	Hg	Hg	
1 C C		13	April 28, 1925 May 15, 1925 May 19, 1925 May 26, 1925 May 28, 1925	Clear	—	71.0	—	5.66	3.34	69.1	106.70	No edema		
				Opalescent	—	71.1	24							
				Slightly opalescent	64.8	71.2	24							
				Opalescent	65.8	71.5	22							
				Clear	64.7	69.2	24							
2 A S		29	April 26, 1925 May 1, 1925 May 12, 1925 May 19, 1925	Clear	—	71.1	—	6.26	1.35	1.69	136.92	No edema		
				Slightly opalescent	—	72.1	24							
				Clear	—	72.5	24							
				Clear	64.0	72.8	24							
				Clear	—	69.0	—							
3 D G		29	April 28, 1925 May 12, 1925 May 19, 1925 May 22, 1925	Clear	—	69.0	—	4.16	2.21	57.9	110.76	Some edema		
				Slightly opalescent	—	66.9	24							
				Clear	61.5	67.8	24							
				Opalescent	61.4	67.7	24	4.31	2.22	58.1		More edema		
				Opalescent	61.4	67.7	24							Edema stationary

4	E. A.	29	18	May 11, 1925	Slightly opalescent	—	70 2	24	47 1	150/80	No edema
				May 18, 1925	Slightly opalescent	65 6	72 2	23	6 60	4 36	33 3
5	V M.	7	12	April 1, 1926	Opalescent	—	69 4	24	5 63	3 26	65 5
									110/80		No edema
6	R. G.	12	13	February 26, 1926	Creamy	—	64 5	22	4 14	1 65	37 5
				March 29, 1926	Creamy	—	63 6	21		47 8	120/90
7	N O	3	16	April 1, 1926	Clear	—	67 6	23	5 80	1 69	21 7
									110/66		No edema

\*The normal value of the concentration index is above .35

Certain relationships are apparent from table 1 and chart 1. Cases 1, 2, 4 and 5 form a group characterized by normal surface tension (64.0 to 65.8 dynes for undiluted serum and 69.2 to 72.8 dynes for serum diluted 1 to 100), by relatively clear sera, slightly lowered or normal plasma proteins and no edema. Cases 3 and 7 form an intermediate group with somewhat lower surface tension. Case 6 presents a

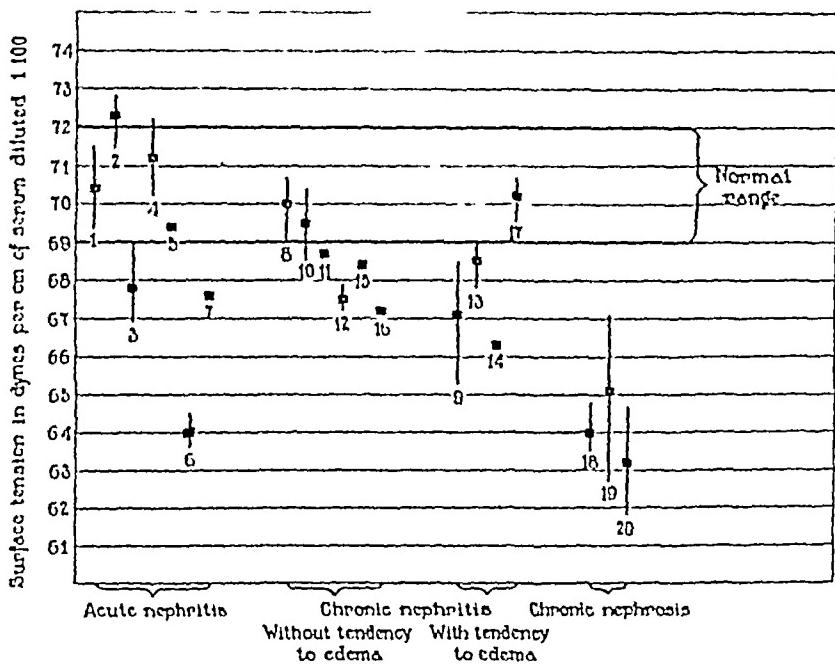


CHART 1. The vertical lines indicate the range of the surface tension values for each case, the solid squares represent the averages, the figures below the lines are the case numbers. Where only a square is shown, only one determination was made.

striking contrast because of its very low surface tension (64.0 dynes for 1/100 serum), the creamy appearance of the serum, and plasma proteins at a concentration of 4.14 per cent. There was temporary absence of edema on a salt-free regime, but edema recurred whenever the smallest amount of salt was added to the diet. In this child there has been a chronic mastoid infection for years to which the nephritis appears to have been secondary. However, this is definitely not a

case of pure nephrosis, because microscopic hematuria persisted and there have been periods of impaired renal function.

*Chronic nephritis.* It is difficult to divide this series (table 2, chart 1), into groups like those of the preceding series, because considerable overlapping occurs. Cases 8 and 10 have normal surface tension values, clear sera, moderately diminished plasma proteins, and no edema. Cases 9, 11, 12, 13 and 14, form a motley group, the first and last showing much edema and fluid in the serous cavities, while the other cases were free from edema. All degrees of renal functional impairment were represented. The surface tension varied extensively both in the group and in the individual cases. No correlation could be made out between the surface tension and the level of plasma proteins. Case 13 showed the exceptional occurrence of a milky or creamy serum with almost normal surface tension. This was the only case with this combination and no explanation can be offered. Case 15, a diabetic with chronic nephritis, and case 16, a gouty patient with chronic nephritis, are grouped together because they showed the unusual combination of relatively high plasma protein figures and moderately lowered surface tension, without edema. Case 17 was originally diagnosed as chronic nephrosis, but later developed the typical features of chronic nephritis. This patient had clear serum, which is an unusual finding in a case of nephrosis. She furnished the most interesting exception of all, normal surface tension in the presence of non-cardiac edema and extremely low plasma protein values.

While usually a tendency to edema, low plasma albumin content, and high plasma fat content are associated with lowered surface tension, combinations occur which are strikingly at variance with the assumption that these features occur consistently together. We have observed creamy serum with but slightly subnormal surface tension, high plasma proteins with abnormally low surface tension, very low proteins and normal surface tension, and considerable edema or tendency to edema with normal surface tension.

*Chronic lipoid nephrosis.* The three cases in this series (table 3, chart 1) fall naturally into one group, marked by milky or creamy sera, varying amounts of edema and fluid in the serous cavities, low plasma protein concentrations and consistently low surface tension. Yet the

## SURFACE TENSION IN NEPHRITIS

TABLE II  
The surface tension of the serum in chronic nephritis

Case number	Name	Age	Date	Appearance of serum	Surface tension of serum		Plasma proteins		Blood pressure		Remarks
					Diluted 1/100	Diluted	Tc°	Fc°	Urea concentration	Urea	
8	M M. C	19 years	May 1, 1925	Clear	—	70.3	24	5.57	3.60	12.7	148/86 No edema
			May 2, 1925	Clear	—	69.0	24	—	—	13.7	
			May 19, 1925	Clear	65.0	70.3	24	—	—	17.9	
			May 22, 1925	Clear	64.8	69.8	24	5.93	1.02	—	
			May 26, 1925	Clear	64.0	70.7	22	—	—	21.9	
9	A C	26 years	May 11, 1925	Clear	—	66.0	24	4.12	1.64	36.2	124/92 Much edema and hydrothorax
			May 18, 1925	Clear	—	65.3	23	—	—	45.9	
			May 25, 1925	Opalescent	61.0	67.8	22	—	—	31.0	
			March 22, 1926	Opalescent	—	68.4	21	—	—	13.7	
			April 1, 1926	Slightly opalescent	—	66.8	24	4.72	1.71	11.3	
			April 19, 1926	Slightly opalescent	62.3	63.5	23	—	—	15.7	
10	E S	29	May 11, 1925	Clear	—	68.5	24	—	—	7.36	196/120 No edema
			May 15, 1925	Clear	—	70.1	24	5.26	2.99	9.08	
			March 22, 1926	Clear	—	69.5	22	5.26	2.99	5.68	
			March 22, 1925	Slightly opalescent	62.0	68.7	24	4.29	2.34	33.6	
11	J L	16	—	—	—	—	—	—	—	—	142/80 No edema

12	G O	48	February 19	1926	Clear	—	67 2	23	5 11	3 35	7 54	246/144	No edema
13	R. V.	25	February 23	1926	Milky	—	67 8	22	17 5	154/78			No edema
			March 4, 1926		Milky	—	69 0	22	17 4				
			March 29, 1926		Creamy	—	68 6	21	4 13	2 22	19 9		
14	F C.	24	April 1, 1926		Clear	—	66 3	23	4 16	1 44	22 7	118/88	Much edema and ascites
15	M. O.	45	March 4	1926	Opalescent	—	68 4	23	6 59	3 54	9 44	164/94	No edema, diabetes
16	L W	37	April 1, 1926		Clear	—	67 2	23	7 15	3 69	11 3	165/110	No edema, gout
17*	B F	23	April 27, 1925		Clear	—	69 5	—	3 55	1 45	19 1	96/64	Moderate edema
			May 18, 1925		Clear	64 0	70 4	23			18 8		
			May 25, 1925		Clear	64 0	70 7	22	3 82	1 80	16 8		

\* This case began apparently as nephrosis and was also suspected of having Addison's disease.

TABLE 3  
The surface tension of the serum in chronic lipoid nephrosis

Case number	Name	Age	Date	Appearance of serum	Surface tension of serum		Room temperature	Plasma proteins	Urea content index	Blood pressure	Remarks
					Undiluted	Diluted 1:100		Total	Albu min	$\frac{U}{B} \sqrt{\frac{V}{W}}$	
18	W J	27	April 27, 1925	Creamy	—	63.5	—	3.86	1.65	55.8	Slight edema Edema increasing Moderate stationary edema
			April 30, 1925	Milky	—	64.8	23				
			May 2, 1925	Creamy	—	63.7	24				
			April 28, 1925	Creamy	—	62.7	—	4.03	1.79	34.7	
19	M R	29	April 30, 1925	Creamy	—	64.1	23				Some edema on calcium chloride therapy
			May 12, 1925	Creamy	—	64.0	24				
			May 19, 1925	Creamy	60.0	67.1	24				
			May 26, 1925	Creamy	61.8	67.0	22	4.10	2.19	31.5	
			May 28, 1925	Creamy	61.0	66.0	24				
			March 8, 1926	Opalescent	—	64.7	24	4.46	1.22	19.0	Edema, much ascites and hydrothorax
			April 19, 1926	Creamy	56.6	61.8	23				
20	D B	9									Edema and much ascites

presence of all four features does not necessarily denote a *pure* lipoid nephrosis, e.g., case 6 in table 1

*The time-drop phenomenon of serum in renal disease.* DuNoüy (6) found that when serum was allowed to stand exposed to air and the surface tension was measured at intervals, a distinct fall occurred. The explanation for this spontaneous fall in surface tension lies in the accumulation of surface active substances, as a function of time, at the surface layer of the liquid, and in the possible formation of new surface-active material from substances present in the serum. A similar phenomenon occurs in aqueous solutions of sodium oleate and other organic substances. The fall amounted to only a few dynes in the case of undiluted serum, but to as much as 10 to 15 dynes or more when the serum was highly diluted, for example 1:10,000. The fall in surface tension begins as soon as the surface has been formed, is very rapid in the first few minutes, is almost completed within 20 minutes and reaches an equilibrium at the end of two hours. If the surface of the liquid is then disturbed, as by stirring, the surface tension rises again, to be followed by another time-drop. The extent of the difference between the initial value of the surface tension and that at the end of two hours varies with the dilution of the serum. The time-drop increases with increasing dilution until an optimum dilution is reached, and then diminishes progressively with increasing dilution.

In this work the sera were diluted with 0.9 per cent NaCl, 1:100, 1:1,000, 1:5,000 and 1:10,000. The surface tension was measured at once and at the end of two hours, the liquid being left undisturbed during the interval. The difference between the two readings constitutes the time-drop. The maximum time-drop occurred usually at a dilution of 1:5,000 or 1:1,000.

Table 4 shows the results obtained on different sera in the various types of renal disease. The great variation in the results, even for the same individual, contrasts with the relatively constant figures for surface tension in the preceding tables. There is also an entire lack of any outstanding difference between the values for one type of renal disease and those for another. It is true that the sera with low initial surface tension, as in cases 9, 11, 18 and 19, tend to have greater time-drop values in the various dilutions than the sera with normal initial surface tensions, but a single determination may fail to show this,

TABLE 4  
The time drop in the surface tension of dilute serum in renal disease

Case number	Name	Date	Fall in surface tension of serum in 1 hour at 37° C. centimeter when the serum was diluted				Diagnosis
			1:100	1:1000	1:5000	1:10,000	
1	C C	April 28, 1925	9.0	15.0	—	13.9	Acute nephritis
		May 15, 1925	7.6	13.7	15.8	13.3	
		May 19, 1925	6.7	—	10.6	—	
		May 26, 1925	7.5	12.2	10.3	7.0	
		May 28, 1925	—	10.8	11.5	—	
2	A S	April 28, 1925	9.6	16.4	—	11.3	Acute nephritis
		May 1, 1925	10.7	19.1	17.6	10.8	
		May 12, 1925	8.1	14.2	11.4	11.0	
		May 19, 1925	8.2	—	9.2	—	
		—	—	—	—	—	
3	D G	April 28, 1925	9.0	15.6	—	5.9	Acute nephritis
		May 12, 1925	6.7	16.2	11.1	9.4	
		May 19, 1925	8.6	—	13.1	—	
		May 22, 1925	7.3	18.6	20.8	—	
		—	—	—	—	—	
4	E A	May 11, 1925	7.2	13.5	17.0	12.0	Acute nephritis
		May 18, 1925	6.5	16.0	17.5	15.8	
		—	—	—	—	—	
		—	—	—	—	—	
8	M McC	May 1, 1925	10.0	18.5	18.7	16.5	Chronic nephritis
		May 2, 1925	6.8	14.4	17.5	14.2	
		May 19, 1925	6.6	—	12.1	—	
		May 22, 1925	6.1	18.8	18.9	—	
		May 26, 1925	6.7	11.4	11.5	9.1	

9	A C	May 11, 1925	6 6	17 3	17 8	13 0	Chronic nephritis
		May 18, 1925	10 1	21 8	19 9	16 8	
		May 25, 1925	8 6	15 9	12 2	8 4	
		May 15, 1925	6 9	14 2	17 6	14 1	Chronic nephritis
10	E. S.	May 11, 1925	6 6	15 7	17 1	13 0	
		May 22, 1925	7 9	19 5	20 6	—	Chronic nephritis
		April 27, 1925	5 5	13 2	11 8	6 1	
		May 18, 1925	7 7	20 6	19 7	12 2	Chronic nephritis
11	J L.	May 25, 1925	9 2	11 5	8 7	5 3	
		April 27, 1925	14 1	20 2	24 8	—	Chronic lipoid nephrosis
		April 30, 1925	7 2	14 3	12 7	8 4	
		May 2, 1925	10 7	19 8	23 6	16 7	
12	W J.	April 28, 1925	9 0	10 3	—	—	Chronic lipoid nephrosis
		April 30, 1925	7 5	13 9	14 5	12 0	
		May 12, 1925	10 3	20 1	15 8	13 5	
		May 19, 1925	14 4	—	17 4	—	
13	M. R.	May 26, 1925	9 0	17 1	12 5	10 5	
		May 28, 1925	12 3	18 6	18 2	16 2	

and when frequent measurements are made, high time-drop figures may appear in cases with normal initial surface tension. Finally, while the time-drop in dynes is greater at a dilution of 1 1,000 or 1 5,000 than at 1 100, one can, for comparative purposes, use the figures on 1 100 serum and thereby save time and glassware. It was only in cases 18 and 19, both nephrosis, that the time-drop in two hours exceeded 10 dynes in the case of 1 100 serum.

#### GENERAL CONSIDERATIONS

*Low surface tension of the serum and edema.* If one were to find a constant relationship between low surface tension of the serum and the presence of edema or a tendency to edema it would be tempting to explain edema as due to the effect of a surface-active substance. Its action would presumably be on the endothelial wall of the capillaries and would result in an alteration of permeability. Clausen (1) has been able to treat collodion membranes with the plasma or the urine or a surface-active, wax-like substance isolated from the urine of patients with parenchymatous nephritis with the result that the collodion membranes originally impermeable to protein become permeable. However, edema has not been experimentally produced by means of this surface-active substance. Furthermore, there is no clear evidence that an alteration in permeability of cellular membranes plays a rôle in the production of nephrotic transudates. The term "permeability" is used here only in reference to the passage of proteins through cell membranes. Possibly the edema fluid in acute glomerular nephritis is an exudate containing an appreciable amount of protein. This is not the case, however, in chronic nephrosis. Yet, it is in the latter that the lowest serum surface tension values are found.

In general, patients who have edema, or a strong tendency to edema, also have a lowered surface tension of the serum. However, there are a number of exceptions even in our relatively small group of cases. Case 17 (table 2 and chart 1), had normal surface tension in the presence of considerable edema. Case 9 (table 2 and chart 1) had almost normal surface tension on two occasions when edema was present. The development of edema in our cases is evidence of a marked tendency to edema because the patients had been on a salt-free diet and a restricted fluid intake for considerable periods of time. Case

13 (table 2 and chart 1) with but slightly subnormal surface tension, showed at first no edema but had evidently a tendency to edema for he developed edema rapidly when allowed to take salt in his diet. The plasma protein and the plasma albumin figures in cases 17, 9 and 13 showed a far closer parallelism with the tendency to develop edema than did the surface tension values. It is difficult to draw any other conclusion from this fact than that drawn by Govaerts (2), —that low osmotic pressure of the plasma proteins is the constant accompaniment and important cause of non cardiac edema in nephritis.

*Low surface tension and the turbidity of the serum.* The milky or creamy appearance of the serum, so characteristic and constant in many of the cases of renal disease, seems to be usually associated with low surface tension values. Case 13, however, is a distinct exception. Milder grades of lowering of the surface tension may occur with perfectly clear serum. It is natural to think of the high total fat content of these creamy sera as being possibly responsible for the low surface tension.

The correlations between low plasma proteins, high plasma fats and low surface tension present a field for future study rather than for present conclusions. Probably some general metabolic disturbance underlies all three as a common etiological factor.

#### SUMMARY

In the three cases of apparently pure lipoid nephrosis observed, the surface tension of the serum was abnormally low. In the nephritics of other types it varied without apparent relation to the tendency to edema or to any other observed factor (see chart 1).

Tendency to non cardiac edema in nephritis was found associated regularly with lowered plasma albumin content, but only irregularly with lowered serum surface tension.

#### BIBLIOGRAPHY

- 1 Clausen, S W J Biol Chem, 1924, lxi, p xlvi Studies in Parenchymatous Nephritis.
- 2 Govaerts P Bull Acad Roy Med Belgique, 1924, lv, 161 Recherches cliniques sur le rôle de la pression osmotique des protéines du sang dans la pathogenie des oedèmes et de l'hypertension artérielle.

- 2a Goverts, P Compt rend Soc Biol, 1925, xciii, 44 Influence du Rapport Albumines—Globulines sur la Pression Osmotique des Proteines du Serum
- 3 Kisch, und Remertz, O Internat Zeit phys chem Biol, 1914, 1, 354
- 4 Traube, J Internat Zeit phys chem Biol, 1914, 1, 389
- 5 Clausen, S W Amer Jour Dis Child, 1925, xxix, 594 Parenchymatous Nephritis III The Surface Tension of the Blood Serum
- 6 du Nooy, P L Jour Exp Med, 1922, xxxv, 707 Surface Tension of Serum II Action of Lime on the Surface Tension of Serum Solutions
- 7 du Nooy, P L Jour Gen Physiol, 1918-1919, 1, 521 A New Apparatus for Measuring Surface Tension
- 8 du Nooy, P I Jour Gen Physiol, 1921, vi, 625 Surface Tension of Serum XI An improvement of the Technique for Measuring Surface Tension
- 9 Van Slyke, D D, Linder, G C, Hiller, A, Leiter, L, and McIntosh, J F Jour Clin Inv, 1926, n, 255 The Excretion of Ammonia and Titratable Acid in Nephritis

# THE PHYSIOLOGICAL RESPONSE OF THE CIRCULATORY SYSTEM TO EXPERIMENTAL ALTERATIONS

## III THE EFFECT OF AORTIC AND PULMONIC STENOSES

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### INTRODUCTION

"The heart and blood vessels place themselves in harmony with an increased volume flow of blood through them, the former by dilatation<sup>1</sup> and hypertrophy and the latter by dilatation" (1, 2) This concept was established experimentally by the production of peripheral (3) and intracardiac arteriovenous fistulae (1) In the presence of such fistulae there occurred a rapidly developing enlargement of the heart, demonstrable both by the roentgenogram and by postmortem examination This enlargement of the heart consisted primarily of dilatation, and secondarily of hypertrophy The enlargement was proportional to the size of the fistula, which determined the volume flow of blood through the abnormal communication and, therefore, through the heart

The following studies were undertaken to determine the effect of altering the flow through the heart by introducing into the circulatory system a pulmonic stenosis or an aortic stenosis, and to compare the effect of such stenoses with the effect of a defect in the interventricular septum It may be considered that the effect of such stenoses is to impede the flow of blood through the circulatory system by increasing the peripheral resistance against which the right and left ventricles perform their work while the effect of the septal defect is to increase the flow of blood through the heart

<sup>1</sup> Dilatation as used here refers to an enlargement of the intracardiac cavities due to distention and not to a pathological state of the cardiac musculature.

## EXPERIMENTAL METHODS

The heart was exposed at operation under aseptic precautions. Ether anesthesia was used. Mechanical respiration was provided by the Erlanger double cylinder. Stenoses of the aorta and pulmonary artery were produced in dogs by placing around the vessel a constricting band of tape or aluminum (4). Interventricular fistulae were produced by incising the septum with a long slender knife inserted through the left ventricle.

During the period of observation the pulse rate and the presence of thrills and murmurs were noted. The volume of the circulating blood was determined by the intravenous injection of brilliant vital red. Changes in the size of the heart were determined by roentgenograms and at autopsy the heart was measured and the cardiac muscle was studied histologically.

## DISCUSSION

Certain observations in the protocols which follow may be emphasized. A stenosis which permanently constricted the pulmonary artery to a circumference less than one-half the normal size was invariably fatal. A pulmonic stenosis of marked degree was immediately followed by an acceleration in pulse rate and by a drop in general blood pressure, both of which gradually approximated normal. The recovery of blood pressure was more rapid in the presence of a pulmonic stenosis than in the presence of a large interventricular septal defect (1). The stenosis was followed immediately by a dilatation of the right ventricle (experiments 6 and 9), a dilatation which gradually subsided during the subsequent months until the heart again assumed a normal size as shown by roentgenographic studies (experiment 4). At necropsy (experiment 4) a slight thickening of the right ventricular wall was found as previously observed by Reid (5). Microscopically, the right heart showed evidence of hypertrophy definitely more marked than in the left heart. The slight effect of a pulmonic stenosis of this duration upon the size and thickness of the right ventricle was in marked contrast to the pronounced effect of a large interventricular defect of equal duration as observed in our previous study (1). The latter was followed by a conspicuous dilatation and thickening of the

wall of the right heart. There was no demonstrable increase in total blood volume after pulmonic stenosis such as developed in the presence of interventricular defects.

The production of the pulmonic stenosis resulted in a well marked systolic bruit followed by an accentuated pulmonic second sound. A thrill was felt at the time of operation limited to the vessel beyond the stenosis. It could not be felt by palpation of the chest wall, whereas the septal defect invariably produced a pronounced thrill palpable over the chest wall.

The establishment of an interventricular septal defect some days after the production of a pulmonic stenosis in the same animal resulted, when the animal survived, in a relatively much greater enlargement of the heart from the septal defect, as compared to the slight enlargement which followed the production of the pulmonic stenosis alone (experiments 10, 11 and 12). This enlargement of the heart is due almost entirely to dilatation rather than to hypertrophy. It may be inferred, therefore, that an increase volume flow through the right heart resulting from a septal defect is a more effective means of producing right sided cardiac enlargement than is the increased peripheral resistance resulting from a pulmonic stenosis.

Additional evidence was also obtained indicating that in the presence of interventricular defects there is an increased flow of blood through the pulmonary circulation and that this may be diminished by producing in the same animal a pulmonic stenosis. In experiments 10 and 11, the animals survived large septal defects when associated with a pulmonic stenosis, whereas interventricular defects of equal or of smaller size in other animals proved invariably fatal due to pulmonary congestion and edema. That this pulmonary congestion and edema which accompanies excessively large interventricular defects may be the important factor in the death of these animals was indicated by the turn of events in experiment 10 following erosion of the constricting tape through the pulmonary artery. This erosion permitted an increased flow through the pulmonary circuit producing marked congestion and edema of the lungs which proved fatal.

Aortic stenosis beyond the left subclavian artery lowered peripheral blood pressure but caused no acceleration in pulse (experiments 3 and 1). Such a stenosis not only lowered femoral pressure but increased

carotid pressure. Well marked aortic stenosis of six months' duration produced no roentgenographic increase in the size of the heart, and at necropsy there was no apparent dilatation of the heart, but a slight increase in thickness of the left ventricular wall, which microscopically showed a slight hypertrophy.

An instructive comparison may be made between experiment 1, in which an aortic stenosis of six months' duration was produced, and an experiment already published, in which a large septal defect of eight months' duration had been produced. These animals were of the same breed and weight, but at necropsy the heart of the former animal weighed 165 grams, whereas the heart of the latter animal weighed 226 grams. It is quite evident that the septal defect with its resulting increased flow of blood through the heart produced a much greater hypertrophy of the cardiac muscle than did an aortic stenosis sufficient to cause a permanent lowering of the peripheral femoral pressure. We may infer, therefore, that *an increased flow of blood through the heart is a more effective stimulus to cardiac dilatation and hypertrophy than increased peripheral resistance*. The total blood volume remained relatively unchanged in the presence of an aortic stenosis. The production of an aortic stenosis was followed by a systolic bruit heard best along the left vertebral border. There was no palpable thrill.

Certain other differences may be noted in the effects produced by large interventricular septal lesions as compared to the effect of a pulmonic or aortic stenosis. In the former there were invariably microscopic and macroscopic evidences of pulmonary congestion and edema. In the latter these were not consistently observed either macroscopically or microscopically, and in most of the cases of uncomplicated pulmonic stenosis a marked emphysema was noted (experiments 6, 7 and 8). In those animals with an aortic stenosis in which a sudden exsanguination occurred through erosion of the vessel wall there was also microscopic emphysema of the lungs. Respirations were more rapid in the presence of an increased flow through the lungs as produced by interventricular defects than in the presence of a pulmonic stenosis.

It is evident also that, roentgenographically, a marked enlargement of the heart usually indicates dilatation. Hypertrophy, if it exists,

produces little evidence of its presence by the roentgenograms (experiments 1 and 4) One may infer, therefore, that any great increase in the size of the heart observed clinically is in greater part due to dilatation and in lesser degree to hypertrophy

Partial constriction of the pulmonary artery or aorta by a metal band or tape frequently ended fatally through erosion of the vessel wall

#### PROTOCOLS OF EXPERIMENTS

##### *Aortic stenosis*

*Experiment 1 (dog 143)* Male, weight 20 kilos. Before operation the blood pressure was 170/80, pulse rate 116, and the total blood volume 1800 cc On March 6, 1925, the aorta was constricted to one half its normal diameter by a broad tape at a point just beyond the left subclavian artery A marked thrill could be felt beyond the constriction for about 10 to 12 cm There was no thrill proximal to the tape. The pulse rate at the end of the operation was 132 A loud systolic bruit was audible along the left vertebral border posteriorly, but not elsewhere The femoral artery was easily compressible and the blood pressure could not be recorded.

On March 7th the pulse rate was 108 The systolic pressure in the femoral artery was about 90, and the diastolic pressure could not be determined. On March 10th the pulse rate was 84, and the systolic pressure in the femoral artery was 90 On April 21st the pulse rate was 105, and the blood pressure in the right femoral artery had risen to 115/65 A total blood volume of 1940 cc. was determined. On July 18th the pulse was 114, the blood pressure in the right femoral artery was 120/60 and a blood volume of 1930 cc was determined

On September 4th the blood volume was 1710 cc., the pulse was 129, and the femoral blood pressure according to the Pachon apparatus was 140/60 A well marked systolic bruit was still audible along the left vertebral border The animal was anesthetized with ether, the right femoral and right carotid arteries were cannulated and blood pressure readings were obtained with a mercury manometer The femoral blood pressure varied with respirations from 136 to 142, corresponding well with the readings of the Pachon apparatus Simultaneous readings of the blood pressure in the carotid artery varied with respirations from 168 to 180 Normally the femoral pressure in dogs is recorded as being higher than the carotid pressure The lower femoral pressure in this instance is ascribed to the degree of aortic stenosis present.

The animal was killed. The heart appeared normal in size, the liver was large and weighed 830 grams the lungs were everywhere normal and weighed 230 grams The heart was opened, there was no dilatation of its cavities (fig 1), but the left ventricular wall appeared slightly thickened The heart weighed 165 grams

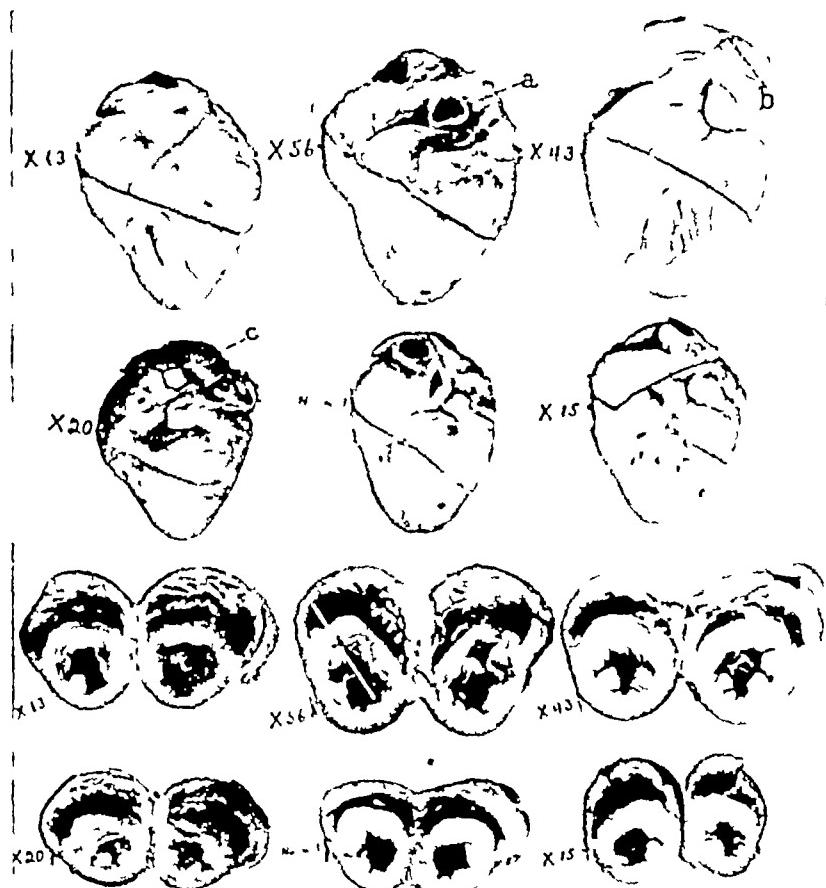


FIG 1 HEARTS REMOVED AT NECROPSY

Experiment 9 (dog X63) Pulmonic stenosis of 35 days' duration Slight dilatation of right ventricular cavity

Experiment 10 (dog X56) Pulmonic stenosis of 62 days' duration and interventricular septal defect of 19 days' duration in the same animal Constricting tape *a* eroded through wall of pulmonary artery into its lumen Marked dilatation of both ventricular cavities

Experiment 1 (dog X43) Aortic stenosis at *b* of 6 months' duration No dilatation Slight if any hypertrophy of left ventricular wall

Experiment 4 (dog X20) Pulmonic stenosis at *c* of 6½ months' duration Dilatation of right ventricular cavity with slight hypertrophy

Experiment 5 (dog X15) Pulmonic stenosis of 30 days' duration showing dilatation of right ventricle

Roentgenographic studies during life had shown no demonstrable increase in the size of the cardiac shadow<sup>2</sup>. The aorta at its emergence from the heart measured 40 mm. in circumference and at the point of stenosis 20 mm. in circumference. The pulmonary artery measured 44 mm. in circumference. There was no dilatation of the aorta either proximal or distal to the constriction.

Microscopic sections revealed slight evidence of hypertrophy in the walls of both chambers of the heart, not at all comparable with that found in the presence of interventricular septal defects.

*Experiment 2 (dog 157)* Weight 9.9 kilos. Preoperative studies revealed a pulse rate of 80 per minute and a blood pressure of 140/50 mm. On June 3, 1925, a constricting figure-of-eight tape was applied to the descending aorta just beyond the left subclavian artery. On June 8th a pulse rate of 84 and a blood pressure of 95/35 were recorded. On June 30th the animal was killed in a fight. At necropsy, the area of constriction was well healed. The heart was small, not dilated, nor were the walls thickened. The aorta admitted a 28 mm. bougie at its origin, and a 10 mm. bougie at the point of constriction. The pulmonary artery admitted a 30 mm. bougie at its origin. The lungs were everywhere air containing and showed no areas of congestion. Microscopic sections showed some emphysema with areas also of partial atelectasis. The liver was normal.

*Experiment 3 (dog 159)* permitted some interesting observations at the original operation. The carotid and femoral arteries were cannulated and connected with mercurial manometers. Complete occlusion of the descending aorta resulted in a fall in femoral pressure from 120 to 0 and rise in carotid pressure from 140 to 174. After only partial occlusion of the aorta there was an immediate rise in carotid pressure which soon subsided. The partial constriction resulted in a slight permanent fall in femoral pressure. The death of the animal prevented subsequent observation.

In *experiment 1 (dog 143)* similar manometric studies were made just before death, six months after the production of an aortic stenosis, and there was still a slight lowering of the femoral pressure as compared to the carotid pressure.

#### *Pulmonic stenosis*

*Experiment 4 (dog 120)* Weight 11.3 kilos. On November 4, 1924, the pulmonary artery was constricted by an aluminum band applied with the Halsted band roller (4). The pulse rate increased immediately from 124 to 220. The dog was obviously ill during the next few days with respirations of 40 to 50 per minute. On November 10th the pulse rate was 196, on November 13th, 172. The blood pressure readings were as follows before operation 250/98, the day

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Standardized roentgenograms were taken by having the target and the film at fixed distances from the heart, the former being placed at a distance of 5 feet from the film and the latter touching the animal. By this method direct comparison of the cardiac shadows was possible.

after operation 152/48. Within twelve days the femoral blood pressure had recovered to 220/91. On April 20th, five months later, the pulse was 140. On auscultation a snapping pulmonic second sound was heard, preceded by a high pitched systolic bruit.

The roentgen ray studies showed an interesting sequence of events (fig. 2). Immediately after the production of the stenosis, the cardiac shadow was increased,

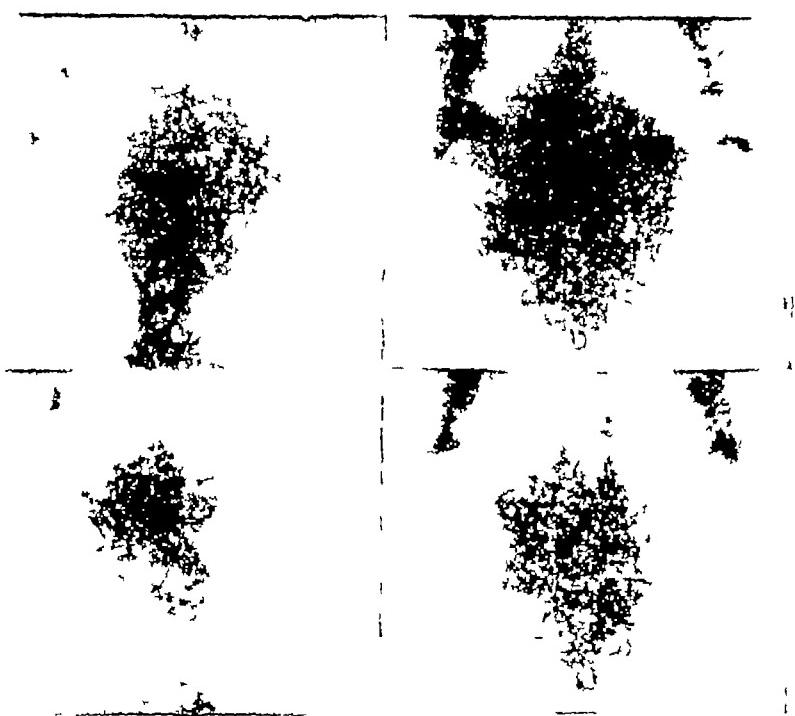


FIG. 2. ROENTGENOGRAMS OF EXPERIMENT 4

*a*, November 4, 1924, before producing pulmonic stenosis, *b*, November 11, 1924, showing definite enlargement due to a prominent right ventricle, *c*, December 26, 1924, enlargement less marked but still present, *d*, February 20, 1925, enlargement has completely subsided

principally by the prominence of the right ventricle. According to the roentgenogram this enlargement persisted for several months and then gradually diminished, so that by February 20th the cardiac shadow had again reverted to the preoperative size.

Blood-volume studies were made. The normal blood volume of a dog weighing 11 kilos is about 1000 to 1100 cc. As the necessary dye was not available, no preoperative studies were possible, but on February 17th, three months after the

production of the stenosis, the animal had a blood volume of 784 cc. A second determination on April 21st indicated a volume of 880 cc. It is apparent that there was no increase in blood volume such as was demonstrated in the presence of interventricular septal defects. The evidence on the contrary, pointed to a small volume that was less than that which normally exists in a dog weighing 11 kg. but no definite conclusions are possible at the present time. An electrocardiogram (fig. 3) revealed a relative preponderance of the right heart.

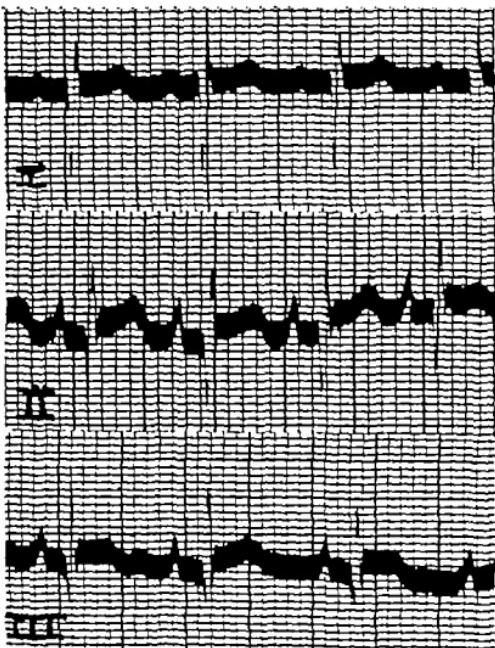


FIG. 3 RELATIVE PREPONDERANCE OF RIGHT HEART IN ELECTROCARDIOGRAM  
EXPERIMENT 4 WITH PULMONIC STENOSIS OF 5 MONTHS DURATION

On May 22nd, following a short illness in which the dog lost weight rapidly, he was found dead. At necropsy, the aluminum band around the pulmonary artery was deeply imbedded in fibrous tissue. The lungs were congested and firm throughout with edema. There were a number of petechial hemorrhages over the surface of the heart and pericardium. The dog had probably died from an acute infection with an associated septicemia. As already indicated by roentgen ray studies made during life, the heart appeared small or normal in size (final weight 117 grams). The pulmonary artery admitted a 20 mm bougie;

site of constriction, the aorta admitted a 32 mm bougie. The right ventricular wall was slightly thicker than normal, being 7 mm wide at its narrowest portion. The right ventricular cavity appeared somewhat larger than the left ventricular cavity (fig 1). The liver was congested, the spleen and kidneys were normal. Microscopic sections showed some congestion and edema of the lungs, with central congestion of the liver. Microscopically the heart showed a slight degree of hypertrophy *more definitely evident in the right heart than in the left*.

*Experiment 5 (dog #15)* Male, weight 11 kilos. On September 24, 1924, the pulmonary artery was constricted by a tape, placed just beyond the pulmonary valve. The diameter of the artery was reduced from 1.7 to 1.2 cm. A thrill could be felt at the time of the operation by direct palpitation of the pulmonary artery and its branches beyond the stenosis. The right ventricle appeared dilated immediately after the application of the tape. The pulse rate increased from 124 to 129. Two days later the pulse rate was 164, respirations were not increased, and there was no cyanosis. Following the operation there was at no time a palpable thrill over the chest wall, but a systolic bruit could be heard followed by an accentuated pulmonic second sound. The animal remained active and well, but was killed in a flight thirty days after the above operation.

At necropsy, the lungs showed a few areas of congestion in the lower lobes, but there was no generalized congestion. The heart was normal except for a slight prominence of the right ventricle and of the conus arteriosus, and on cross-section the right ventricular cavity was dilated (fig 1). The pulmonary artery admitted a 24 mm bougie at the site of the constriction, and the aorta a 34 mm bougie. The heart weighed 88 grams.

*Experiment 6 (dog #19)* Female, weight 13 kilos. On October 24, 1924, an aluminum band 8 mm broad was applied to the pulmonary artery and rolled so as to diminish its diameter by one half. The right ventricle immediately became dilated. The pulse rate increased from 132 to 144 and within a few minutes to 176 rising gradually to 208 at the end of the operation, and to 220 twelve hours later. At this time the animal looked ill, the blood pressure in the femoral artery could not be recorded and respirations were rapid. The animal was found dead thirty-six hours after operation.

At necropsy, the lungs were everywhere normal in appearance. The liver was congested, whereas the spleen and kidneys did not appear congested. The heart showed a marked dilatation of the right ventricle and of the conus arteriosus. On cross section the right ventricular cavity was dilated whereas the left ventricular cavity appeared small. The right auricle was prominent. The aluminum band lay 1 cm above the valves. The pulmonary artery admitted a 38 mm bougie at its emergence from the heart and an 18 mm bougie at the site of the constriction. The aorta admitted a 34 mm bougie. Microscopic sections revealed marked emphysema of the lungs but no edema nor congestion. There was marked congestion of liver and kidney.

*Experiment 7 (dog #22)* Male, weight 12 kilos. On October 30, 1924, a

pulmonic stenosis was produced as in experiment 6. The pulse rate increased from 148 to 196. The animal became very ill with rapid though shallow respirations and was found dead forty-eight hours later. At necropsy, the right ventricle was dilated. The stenosed pulmonary artery admitted an 18 mm. bougie, the aorta a 34 mm. bougie. Microscopic sections revealed emphysema of the lungs, with moderate congestion of the liver.

*Experiment 8 (dog λ48)* Weight 15.7 kilos. On April 21, 1924, the pulmonary artery was constricted to one half its normal diameter by applying a figure-of-eight tape at a point just beyond the pulmonary valves. The dog died twenty two days later from hemopericardium following erosion of the pulmonary artery just proximal to the tape. Microscopically the lungs showed a marked emphysema with some areas of atelectasis. The liver showed marked congestion with slight congestion also of the kidneys. The animal is included in the series because it illustrates a frequent complication attending the partial ligation of large arteries, namely, the erosion of the wall of the vessel by a constricting band, either of metal or of tape.

*Experiment 9 (dog λ63)* Female, weight 18 kilos. Preoperative studies revealed a pulse rate of 116 and a blood pressure of 150/75. On July 15th the pulmonary artery was constricted to one half its normal diameter. There developed a dilatation of the right ventricle and conus arteriosus and an immediate acceleration of pulse rate from 136 to 164. Two days later the animal was breathing normally with a respiratory rate of 32, a pulse rate of 180, and a blood pressure in the femoral artery of 90/50. There was a marked systolic murmur with a definitely accentuated pulmonic second sound. On July 30th the pulse rate was 152 and the blood pressure was 90/40. On August 20th the animal was killed. The right ventricle appeared slightly dilated, and the heart weighed 130 grams (fig. 1). The aorta admitted a 38 mm. bougie, the constricted pulmonary artery a 30 mm. bougie. As determined by the roentgenogram the transverse diameter of the heart measured 6.8 cm. before operation and 7.8 cm. on July 30th, the increased width being due to the dilated right ventricle.

#### *Pulmonic stenosis associated with an interventricular septal defect*

*Experiment 10 (dog λ56)* Male, weight 19.8 kilos. Before operation the pulse rate was 120 and the blood pressure 170/70. On May 11, 1925, a pulmonic stenosis was produced by the application of a tape just distal to the pulmonary valves. When this tape was first applied it was constricted to such a degree that the left auricle collapsed, and the right heart became markedly dilated. It was, therefore, loosened so as to constrict the artery only one half its normal size and with only slight dilatation of the right ventricle. On the next day, the respirations were slow, pulse 153 and blood pressure 140/55. On June 1, 1925, a pulse of 124 and a blood pressure of 140/70 were recorded. There was a well marked systolic bruit followed by an accentuated pulmonic second sound.

On June 24th the animal was anesthetized with ether and the heart exposed

It appeared normal in size. The constricting tape around the pulmonary artery was buried in fibrous tissue. The conus arteriosus was somewhat prominent. A large interventricular defect was established with a knife introduced through the apex of the left ventricle (1). This was followed by a marked systolic thrill over the right ventricle, and an immediate acceleration of pulse from 112 to 168. At the end of the operation the pulse rate was 220. A few hours later, the respirations were 120 and were labored and grunting, the femoral pulse was imperceptible and the heart beat was so rapid it could not be counted. Judging from our previous experiences following the establishment of large interventricular defects, this animal was not expected to live. On the following morning we were astonished to find the dog standing in his cage, having eaten a good breakfast. The respirations were 72 and the pulse rate 171. The blood pressure in the femoral artery was below 90. There was a marked systolic and diastolic thrill over the right side of the chest. On June 25th, the pulse rate was 165, blood pressure 110/60 and respirations 64.

On July 1st, the pulse rate was 148, respirations 52, and blood pressure 130/50. July 13th, quite unexpectedly, the animal was found dead. At necropsy, the mouth was filled with frothy bloody fluid. There was no free fluid either in the abdomen or in the thorax, and no congestion of liver, spleen or kidneys. The lungs, however, were blue black in appearance with a consistency like that of liver and with no air containing areas. The lungs weighed 780 grams, whereas the liver weighed only 680 grams. The bronchi were filled with frothy bloody fluid. The heart was large and appeared dilated. On cross section both ventricular cavities were large (fig. 1). The interventricular defect admitted a 34 mm bougie. The aorta admitted a 36 mm bougie, and the pulmonary artery admitted a 30 mm bougie at the site of constriction. The constricting tape was found lying within the lumen of the pulmonary artery having eroded its way through the wall of the artery. Perforation into the thoracic cavity had been prevented by the thick fibrous deposits around the tape.

This animal died with a marked pulmonary edema and congestion, and exhibited at necropsy the same picture noted in those animals in which excessively large interventricular defects had been established. In these latter animals it was found that a defect larger than 30 mm was invariably fatal. This animal, however, withstood a defect of 34 mm for nineteen days. The sequence of events may be interpreted as follows. During the time following the second operation in which the constricting tape prevented an excessive volume flow of blood through the lungs, the animal remained well. In fact, his survival with so large a septal defect may be definitely attributed to the presence of the constriction of the pulmonary artery. When the tape eroded through the wall of the pulmonary artery on the nineteenth day, its lumen became larger allowing an excessive amount of blood to flow through the pulmonary circuit, followed by marked pulmonary congestion, edema and death.

The roentgenographic studies were instructive. Before operation the trans-

verse diameter measured 7.4 cm On June 23rd forty two days after the constriction of the pulmonary artery the diameter measured 8.0 cm On July 9th, fifteen days after the establishment of an interventricular septal defect, the diameter measured 9.3 cm., showing a rapid increase in the size of the heart accompanying the septal defect

*Experiment 11 (dog A55)* Male weight 14.8 kilos A preoperative pulse rate of 92 and a blood pressure of 170/75 were determined. On May 14, 1925, the pulmonary artery was exposed and a tape applied so as to encircle it tightly but not to constrict it. The pulse rate rose to 177 An interventricular septal defect was then established The pulse rate increased to 208 There was an intense thrill over the right ventricle It seemed that the flow through the pulmonary circuit was excessive and if allowed to persist would probably end fatally Accordingly, the tape around the pulmonary artery was tightened constricting the artery to a slightly greater degree. The pulse rate fell immediately to 184 and the intensity of the thrill over the right ventricle was diminished The right ventricle was dilated

Four hours later the dog showed labored breathing with a very rapid small pulse which could not be counted We believed the animal would die Twenty four hours later he had taken some milk and was standing in his cage The pulse rate was 160 with a femoral blood pressure of 110 and respirations of 40 The thrill over the right chest had greatly diminished but was still palpable During the next few days the animal was obviously ill with a pulse rate of 180 rapid, labored breathing and a blood pressure of 100 Gradual improvement followed, and by June 2nd the blood pressure had recovered to 115/30 with a pulse rate of 165 There was a definite though circumscribed thrill over the pulmonic area, with a loud systolic bruit, corresponding more to the bruit of a pulmonic stenosis than to that of an interventricular leak On July 9th the animal had a pulse rate of 168 and a blood pressure of 120/50

On July 20th, two months after the operation, the animal was found dead On opening the chest, both thoracic cavities were filled with blood, and examination revealed complete erosion of the wall of the pulmonary artery by the tape, a part of the tape lying within the lumen and a part without. The right ventricle appeared dilated and more capacious than the left The septal defect admitted a 26 mm bougie, the pulmonary artery a 26 mm. bougie and the aorta a 32 mm bougie The heart weighed 156 grams The liver appeared normal and weighed 540 grams The lungs were everywhere soft, air-containing and weighed 180 grams (in marked contrast to the weight of 780 grams in experiment 10) Microscopic sections revealed no edema and no congestion of the lung, but a slight compression atelectasis

Röntgenographic studies before operation showed a heart with a transverse diameter of 8.0 cm , fifty-seven days after the establishment of the combined pulmonary stenosis and interventricular defect the diameter measured 9.1 cm

*Experiment 12 (dog A16)* Male, weight 12.4 kilos On September 25, 1924,

a pulmonic stenosis was produced by means of a constricting tape with an immediate acceleration of pulse rate from 160 to 240. Before operation the pulse rate was 108 and the blood pressure was 196/96. Two days following the operation the pulse rate was 191, blood pressure 136/48 and respirations 36. There was a well marked systolic bruit, but no palpable thrill. The pulmonic second sound was snapping and accentuated. By October 3rd the blood pressure had recovered to 194/80. October 11th a pulse rate of 198 was recorded, and October 30th the rate was 100. There was no visible cyanosis in spite of a well marked pulmonic stenosis.

On December 9, 1921, a septal defect was established, with the immediate development of a palpable thrill over the right ventricle. On the following day, the pulse rate was 177. There was a blowing systolic murmur accompanied by a palpable thrill. On December 24th it was noticed that the dog had a slightly distended abdomen. This rapidly grew worse and was obviously due to an ascites. Three hundred cubic centimeters of clear brownish yellow fluid were removed from the abdomen. The hind legs were markedly swollen. The pulse rate was 172.

On January 7th the dog was examined under ether anesthesia and then killed. The chest contained 1 liter of pink fluid. The lungs were normal, and showed no areas of congestion. The abdomen contained three liters of clear yellowish fluid. The liver was congested, but the other abdominal organs were normal. The heart, which was surrounded by dense pericardial adhesions, appeared very small. There was a slight thrill over the right ventricle. The tape surrounding the pulmonary artery was deeply imbedded in fibrous tissue. The pulmonary artery admitted a 26 mm bougie at the point of stenosis. There was still a small opening in the septum which admitted a 14 mm bougie. The aorta admitted a 34 mm bougie. The heart weighed 94.5 grams. Microscopic sections revealed a slight edema of the lungs, but no congestion, a marked congestion of the liver, and a definite chronic glomerular nephritis, with thickening of the capsule and casts in the tubules.

The roentgenograms presented interesting information (fig. 4). The preoperative transverse diameter of the heart measured 6.5 cm. On November 6th, forty-two days after the constriction of the pulmonary artery, the diameter measured 7.3 cm. On December 23rd, fourteen days after the establishment of the interventricular defect, it measured 9.5 cm. Roentgenograms subsequent to this date showed very obscure shadows of the heart due to the accumulation of fluid in the thoracic cavity. At necropsy, after the development of a very remarkable generalized edema, hydrothorax and ascites, the heart appeared small, certainly much smaller than the roentgenographic evidence of December 23rd had suggested. Is it possible that with the development of the edema and gradual cardiac decompensation the volume flow through the heart decreased with retrogression in the size of the heart? It was quite evident that the establishment of an interventricular defect resulted in a much greater enlargement of the heart than had occurred following the production of a pulmonic stenosis alone.

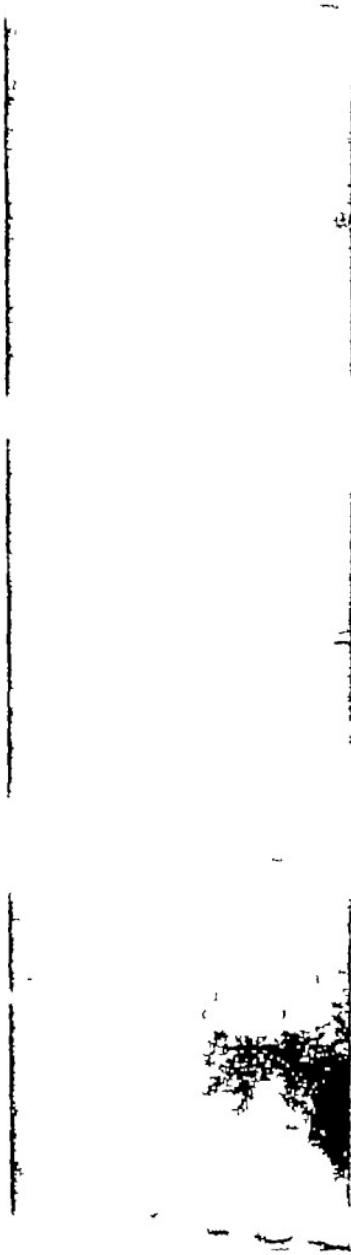


FIG. 4. ROENTGENOGRAMS OF EXPERIMENT 12

*a*, September 24, 1921, before operation, *b*, November 6, 1924, 42 days after production of pulmonic stenosis. Slight enlargement of heart, *c*, marked enlargement of heart on December 23, 14 days after establishing a septal defect in addition to the pulmonic stenosis.

## BIBLIOGRAPHY

- 1 Holman, I., and Beck, C. S. J. Exper. Med., 1925, xlii, 661 The Physiological Response of the Circulatory System to Experimental Alterations I The Effect of Intracardiac Fistulas
- 2 Beck, C. S., and Holman, I. J. Exper. Med., 1925, xlii, 681 The Physiological Response of the Circulatory System to Experimental Alterations II The Effect of Variations in Total Blood Volume
- 3 Holman, I. Arch. Surg. 1924, ix, 556 Experimental Studies in Arteriovenous Fistulas III Cardiac Dilatation and Blood Vessel Changes
- 4 Hilsted, W. S. J. Exper. Med., 1909, xi, 373 Partial, Progressive and Complete Occlusion of the Aorta and Other Larger Arteries in the Dog by Means of the Metal Band
- 5 Reid, M. R. J. Exper. Med., 1924, xl, 289 Partial Occlusion of the Pulmonary Aorta and Inferior Vena Cava with the Metallic Band, Observations on Changes in the Vessel Wall and in the Heart

# THE ASSOCIATION OF GENERALIZED ARTERIOLAR SCLEROSIS WITH HIGH BLOOD PRESSURE AND CARDIAC HYPERTROPHY IN CHRONIC NEPHRITIS

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In the present paper we report observations on the microscopic examination of the vessels of the kidney and other organs in 10 consecutive cases of chronic nephritis in young patients coming to autopsy after previous clinical observation and functional study for some time in the hospital. The data may assist in the eventual clarification of the relationships of renal function and blood pressure changes to cardiac and vascular alterations.

## DEFINITIONS OF PRONOUNCED HYPERTENSION AND OF CHRONIC HYPERTROPHY

The choice of an arbitrary standard by which to decide the presence or absence of pronounced hypertension is a matter of some difficulty, especially since the course of the case in hospital often fails to give a true representation of the whole course of the disease. Fall in blood pressures, (especially systolic pressures) frequently occurs in the terminal stages, even when not attributable to cardiac failure and terminal infection. It has seemed best to use a double criterion, and to class cases as having pronounced hypertension when the systolic pressure has been usually above 180, or when the diastolic pressure has been usually above 110 mm. of mercury.

Normal limits of the heart weight are even more difficult to establish than the limits of blood pressure. Heart weights vary with body weight, sex, age, and nutrition of the individual. We have charted the figures of *absolute* heart weights as given by Müller (1) for males and females of various body weights and in different age periods, excluding cases dying from diseases which are frequently associated

with cardiac hypertrophy, e.g., endocarditis, pericarditis, myocarditis, nephritis, endarteritis and aneurysm, cerebral hemorrhage, chronic pneumonia, pulmonary emphysema, and polyarthritis. We insert one such chart of "normals" as chart 1, the particular chart being the heart weights of males of 50 to 55 kgm body weight. It is obvious that although the mean of the absolute heart weight remains more or

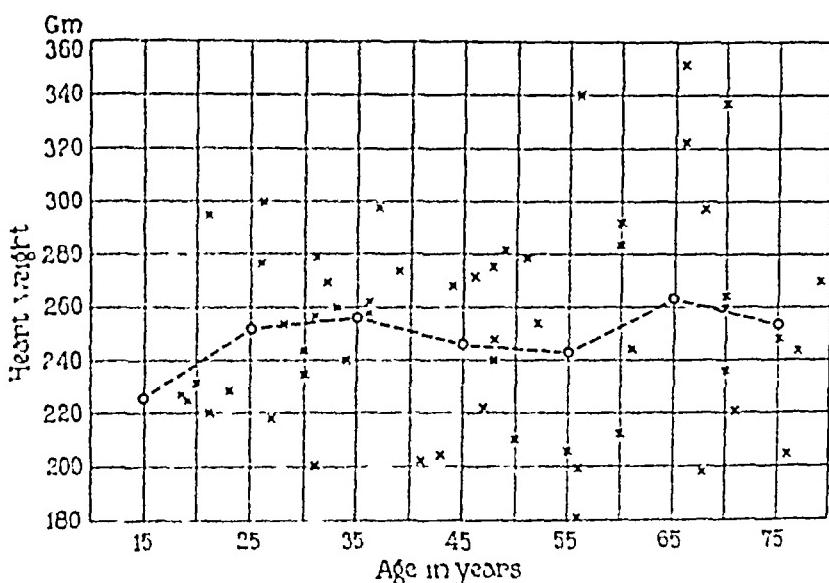


CHART 1 GRAPHIC REPRESENTATION OF MULLER'S FIGURES OF ABSOLUTE HEART WEIGHTS OF "NORMAL" MALES OF 50-55 KGM BODY WEIGHT

× = individual absolute heart weights

○ = mean absolute heart weight in each given age period

less constant in adults of varying ages above 25, the variation of individual observations in each age period is great. From a study of similar charts of males and females of different body weights we concluded that the values, 400 grams for females and 450 grams for males, indicate the upper extremes of normal gross heart weights for individuals of average size, such as were the seven adults in the series of cases reported below. These maxima of gross heart weights are nearly double the average of absolute heart weights observed by Muller. Weights exceeding them may be accepted as indicating

definite hypertrophy, but a weight considerably lower by no means excludes the existence of heart enlargement in the individual

#### THE ANATOMICAL LESIONS OF ARTERIOLAR SCLEROSIS

Sclerosis of the small vessels, first described by Gull and Sutton (2) as "arterio-capillary fibrosis," was later studied and differentiated from sclerosis of the larger vessels by Jores (3), and has more recently received extensive investigation by Evans (4) who proposed for it the term "diffuse hyperplastic sclerosis"

Anatomically, according to Evans, the lesions differ somewhat with the size of the vessel. In the smallest arterioles, such as the "afferent arterioles" of the glomeruli the lesions consist of proliferation of the endothelium of the intima, with hyaline degeneration of the intima, which afterwards frequently shows fatty changes. Narrowing or obliteration of the lumen of the vessel is brought about by these changes. Medial hyperplasia and occasionally perivascular adventitial fibrosis occur. In the vessels from which these smallest arterioles spring, that is in the so-called interlobular arteries of the kidney, those branches of the splenic vessels which run along with the fibrous trabeculae, and in other arterioles of like size, the lesion begins somewhat differently. The first change observed is hyperplasia of the internal elastic membrane, with thickening of the membrane and lamination of the elastic fibres. Hyaline and occasionally fatty changes are to be observed. The lesions occur in the kidney, spleen, brain, retina, liver, pancreas, and occasionally in the appendix. The skeletal muscles and heart are singularly free.

Our observations of these arteriolar lesions accord with Evans' description. In accord with Evans and with Fishberg (5) we have found the lesion most frequently in kidney, spleen, and pancreas, in the order given

#### RESUMÉ OF CASE HISTORIES AND AUTOPSY FINDINGS

(*For details see chart 2 and appended protocols*)

**Cases 1 and 2** Children of 3 years with acute glomerulonephritis passing into chronic glomerulonephritis of the nephrotic edematous type with the nephrotic element predominant. Patients died of intercurrent infections at this stage of the disease. Renal function was still fairly good, as indicated by the blood urea, although probably

CHART 2 SUMMARY OF THE CLINICAL AND PATHOLOGIC

## Clinical and functional findings

Case number	Age	Sex	Edema free weight	Date*	Blood pressure	Phthalein	Blood urea N	Urea concentration index	Blood creatinin mgm per cent
								$\frac{U}{B} \sqrt{\frac{V}{U}}$	
Normal values	years		kgm		mm	per 2 hours per cent	gram per liter	35 or over	Under 1
1	3	F	12	October 20, 1922 October 1, 1923 December 5, 1923 December 7, 1923†	101-80 88-	Over 55	0 202 0 15-0 2		Under 1
2	3	M	16	October 22, 1924 November 7, 1924 December 29, 1924‡	102-64 96-61		0 408 0 090		
3	13	M	40	May 20, 1922 March 9, 1923 October 26, 1923 November 24, 1923 December 4, 1923‡	140-76 114-65 124-70 101-54	45 50	0 21 0 23 0 528	31 8 24 7 6 9	1 3 2 8
4	21	M	55	November 17, 1921 February 10, 1922 March 14, 1922 March 17, 1922‡	206-142 160-110 160-120	0	0 597 1 278 1 145	5 1 2 5	
5	27	F	47	April 14, 1924 May 5, 1924 May 5, 1924‡	160-125 158-117		0 692 2 36	2 9	8 2 20 2
6	34	F	62	May 3, 1923 May 26, 1923 May 27, 1923‡	205-125 135-145	10	1 56 1 5		11 5 25 0
7	28	M	55	October 27, 1923 October 30, 1923 October 30, 1923‡	205-140 205-136		2 19 2 39	1 6 1 6	20 8 23 4
8	27	M	65	November 22, 1923 December 13, 1923 January 9, 1924 January 12, 1924‡	230-125 115-105 202-130	0	1 43 1 39 1 46	2 7	16 5 17 9 17 9
9	33	F	48	December 3, 1923 February 11, 1924 June 19, 1924 July 11, 1924‡	234-100 210-120 228-138	Trace Trace	0 475 0 379 0 964	7 4 5 3	3 97
10	17	M	57 65	October 28, 1921 March 22, 1923 August 8, 1924 August 13, 1924‡	184-122 150-115 112-88	16 8	0 303 0 322 1 70	19 0 8 6	5 0 20 3

\* Dates given are those of phthalein, blood urea N and creatinine determination. Other data in the same line were obtained within 5 days of date given.

† Figures so marked represent plasma CO<sub>2</sub> capacity, expressed in millimols.

‡ Date of death.

## CAL FINDINGS IN THE CASES OF CHRONIC NEPHRITIS

Hemo-globin O <sub>2</sub> capacity per cent	Plasma proteins per cent	Plasma pH	Plasma CO <sub>2</sub> content	Autopsy findings			
				Weight of heart grams	Weight of kidneys grams	Glomerular changes	Distribution of arteriolar sclerosis
17 to 22	6 to 8	7.35-7.46	25-35				
		7.29	8.9	100	220	Scarring, hyalinization, adhesions of tuft to capsule	None
9.1						Congestion proliferation in tuft	None
	3.70 4.59 4.37 3.67			95	170		
				290	430	Hyalinization a few crescents	None
8.7						Ischemia hyalinization fibrosis crescents	None
13.5 10.4	6.32 4.80	7.35 7.28	23.9 22.9	330	180	Ischemia periglomerular fibrosis hyalinization crescents	Kidney spleen pancreas, liver
9.8	6.79	7.27	35.6† 16.7	450	290	Hyalinization fibrosis crescents	Kidney spleen pancreas
13.3 11.3	6.01 5.64	7.32	23.0† 17.2	530	180	Scarring hyalinization crescents	Kidney spleen pancreas
9.4 5.2	5.43 6.28 5.96	7.26 7.18 7.29	15.9 15.5 19.2	490	130	Endoglomerulitis hyalinization ischemia periglomerulitis fibrosis, crescents	Kidney pancreas
7.8 7.6 10.2	5.29 5.71	7.34 7.34	21.8 25.8	555	150	Ischemia hyalinization periglomerular fibrosis, crescents	Kidney spleen pancreas, uterus
18.0 7.4	5.02		15.1 10.7	550	250	Hyalinization, periglomerular fibrosis, ischemia few crescents	Kidney spleen pancreas liver lung

below normal (Unfortunately the difficulty of obtaining from infants urine samples excreted over definite periods prevented the determination of urea concentration indices) Blood pressures were normal Arterioles were normal Heart weights were probably normal for the age of the patients

*Case 3* Boy of 11 Acute glomerulonephritis progressing into chronic glomerulonephritis of edematous type with gradual loss of urea concentrating power Death by intercurrent infection intervened before the functional loss itself reached a fatal degree Blood pressure was normal throughout Arterioles and heart weight were normal at autopsy

*Case 4* Young adult, 21 Chronic glomerulonephritis with edema, ending in uremia about 9 months after first symptoms Renal function was low from the first observation Blood pressure was definitely above normal (160-206 systolic, 110-140 diastolic) throughout observed course Arteries in fundi were tortuous and narrowed, but at autopsy *arterioles were normal in the kidneys* Heart weight, 400 grams, was within normal maximum

*Cases 5, 6, 7, 8, 9, and 10* All young adults with typical advanced, malignant glomerulonephritis, with great loss of urea concentrating power and increase of blood urea concentration All had advanced changes in the fundi All had high blood pressure All died of uremia except case 8, in which cardiac failure caused exstasis At post mortem examination all showed arteriolar sclerosis of kidneys, spleen and pancreas, but not of the muscles All had enlarged hearts except case 5, whose gross heart weight was 330 grams This case progressed very rapidly to death and the arteriolar lesion was of an acute type

#### DISCUSSION

The frequent concurrence of chronic nephritis, hypertension, cardiac enlargement, and arteriolar sclerosis has long been noticed, and has raised discussion concerning the order of incidence and causal relationships of these respective changes However, the difficulty of obtaining evidence even of the order of incidence of the anatomical changes is exemplified by the autopsies on all our patients (nos 4 to 10) who came

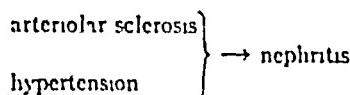
to nephritic deaths. Each patient had hypertension and diffuse glomerular lesions, and all but one had cardiac enlargement. All but one case (no 4) showed also arteriolar sclerosis. By the time nephritis has run its course all these conditions are usually present. It is only by opportunities to examine material from cases of chronic nephritis that have died of intercurrent causes that one is likely to obtain information from which deductions as to the order of incidence can be drawn. Even when such deductions are possible, they permit only inferences concerning the causal relationships. For example, if it were found that hypertension invariably precedes arteriolar sclerosis in nephritis, it would still be uncertain whether the hypertension causes the sclerosis, or whether both are independent results of the nephritis, one developing more rapidly than the other. Our case 4 in which there was fatal nephritis with hypertension but no arteriolar sclerosis, shows that the latter does not in all cases precede hypertension, and hence that some condition in the disease other than arteriolar sclerosis can incite increase in blood pressure. The histories of all these cases are free from evidence of hypertension preceding the nephritis, although it is conceivable that hypertension without subjective symptoms may have been present. As our data stand the apparent interpretation concerning the order of incidence is nephritis, hypertension, arteriolar sclerosis, in this type of rapidly progressing "malignant" hypertensive nephritis.

Evans (4) has come to the conclusion that glomerulitis and arteriolar sclerosis are two separate and independent results of one cause, probably infection. As to the direct cause of the hypertension usually encountered in advanced chronic glomerulonephritis he makes no suggestion, but argues from the small proportion of the arterioles in the body affected by sclerosis (the muscles being exempt) that mere mechanical resistance to the heart pump by contraction of the affected arterioles would be quite insufficient to produce the increase in pressure. With his conclusion that some factor other than arteriolar sclerosis is responsible for hypertension in nephritis our findings in case 4 are in accord.

Fishberg (5) has found that senile hypertension is invariably associated with arteriolar sclerosis. He believes that the hypertension promotes the sclerosis, which is a normal senile result of physiological wear.

and tear, and is merely accelerated by the added strain of hypertension. Concerning the origin of the hypertension he offers no explanation.

The regular association of senile hypertension and arteriolar disease can hardly indicate which one is responsible or whether both arise from a common cause. When renal insufficiency occurs in an elderly subject with hypertension (as it did in about 7 per cent of Fishberg's cases) the order of incidence appears to be



with priority in question between sclerosis and hypertension.

It appears quite possible, as Volhard and Fahr (6) believed, that in one group of nephritics, typified by the young subjects reported in this paper, the nephritis is the initial lesion, exciting the development of hypertension and arteriolar sclerosis, while in another, usually older group, arteriolar sclerosis is the initial lesion, exciting the hypertension, with renal insufficiency eventually developing either from sclerotic strangulation of the glomeruli or from inflammatory processes added to the sclerotic strangulation. In both groups cardiac enlargement presumably follows hypertension.

The hypertension has been conceived as a physiological effort to restore blood flow and function which have been diminished by lesions in either the glomerules or the arterioles of the kidney. However whether hypertension is such a beneficent compensatory effort, or an injurious malfunction, increasing the ill effects of the disease, appears to be still open to debate. Possibly hypertension is necessary for proper renal function in some cases, but it may be so unduly exaggerated that the strain results in cardiac or cerebral death while the renal function is still adequate to support life. That the organism should distribute burdens so equally that the systems among which they are divided all endure for the same maximum period, would be an unreasonable expectation with respect to the capacity for adjustment.

Likewise concerning the causal relationships of concurrent nephritis, hypertension, and arteriolar sclerosis, hypotheses such as we have sketched above are nothing more at present than incomplete state-

ments of problems to be solved Limiting discussion to nephritis of the type reported here (young subjects progressing to chronic, rapidly fatal renal insufficiency), and excluding primary nephrosclerosis with secondary renal insufficiency, the following alternatives appear to be left open for decision from future evidence For cases of the type considered, in which nephritis is apparently the primary cause of the syndrome, either (a) Hypertension may next arise and in turn cause arteriolar sclerosis (all of our hypertension cases might fall within this group), or (b) arteriolar sclerosis may occur and induce hypertension (6 of our 7 hypertension cases might be thus explained), or (c) hypertension and sclerosis may originate independently as a result of the nephritic toxins (This would presumably be the case if either hypertension or sclerosis were found frequently without the other) (d) Finally nephritis may not be the primary cause of the other conditions, but with them the result of a common injurious agent or agents, which incite the development of all three in varying sequence (This would cover all possible findings, but explain the origin of none, until the injurious agents, infectious, metabolic, or other, were identified)

It is quite possible that no generalization can be made, but that the order of incidence and the causal relationships vary, even when the terminal results are similar

#### SUMMARY

1 This report is based on clinical and anatomical findings in 10 young subjects (under 34) with chronic glomerulonephritis. Three cases died of intercurrent infections, 7 came to typical nephritic death, 6 in uremia, 1 from heart failure.

2 The 3 cases (in children aged 3, 3, and 14 years) which terminated by intercurrent infection showed relatively mild but definite, diffusely distributed, glomerular lesions They were free from hypertension, arteriolar sclerosis, or cardiac hypertrophy, and demonstrate that glomerulonephritis can develop to a considerable degree without such results

3 All 7 cases which progressed to typical nephritic deaths showed hypertension and neuroretinitis, together with cardiac enlargement, gross in 5 cases, moderate but probably definite in 2 These findings

accord with the current view that cardiac enlargement in nephritis is the result of hypertension

4 In 6 of these 7 cases arteriolar sclerosis of the parenchymatous organs was found at autopsy. The concurrence of arteriolar sclerosis so frequently with retinitis and hypertension shows that *the presence of hypertension in nephritis indicates usually but not always a co-existing arteriolar sclerosis*

5 The non-conforming case (case 4) confirms Volhard and Fahr (5, p 41) in showing that arteriolar sclerosis may be absent even when neuroretinitis and hypertension have existed for several months, accompanied by renal insufficiency advancing to uremia

#### BIBLIOGRAPHY

- 1 Müller, Wilhelm Die Massenverhältnisse des Menschlichen Herzens Hamburg und Leipsiz, (Voss) 1883
- 2 Gull, W., and Sutton, B. Proc New Sydenham Soc, 1894, 325
- 3 Jores, L. Wesen und Entwicklung der Arteriosklerose Wiesbaden Bergmann, 1903
- 4 Evans, G. Quart Jour Med, 1921, xiv, 260 A Contribution to the Study of Arterio Sclerosis, with Special Reference to its Relation to Chronic Renal Disease
- 5 Fishberg, Arthur, M. Arch Int Med, 1925, xxvi, 650 Anatomic Findings in Essential Hypertension
- 6 Volhard, F., and Fahr, Th. Die Brightsche Nierenkrankheit Berlin, Springer, 1914

#### OUTLINE OF CASE HISTORIES AND AUTOPSY FINDINGS<sup>1</sup>

*Case 1 Hospital No 4001* Female, 3 years old. She had suffered from frequent "colds". Loss of appetite and of good nature began in May, 1922, also swelling of face and extremities. The urine was dark and diminished in amount. Nephritis was diagnosed. Ascites became pronounced, and required tapping. After 12 weeks at home she was taken to the Brooklyn Hospital, where repeated tappings were necessary. Eight weeks later she was transferred to this Institute. She was moderately comfortable. There were enlarged tonsils. The abdomen was immensely distended, and there was edema of the legs and sacral region. The

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<sup>1</sup> The histories and tabulation of functional data have in part been completed during the absence of both the authors. The verification and completion of data from the hospital records, and the corresponding final version of the text, have been undertaken by Dr J. G. McIntosh.

heart was negative. Blood pressure, 102-80. Blood urea and phthalein excretion were normal. The urine contained albumin to the extent of 12 grams per liter. There were granular and hyaline casts, numerous white cells, and very few red cells in the centrifugings. The Wasserman reaction was negative. For 4 months she required frequent abdominal paracentesis. Then reaccumulation of fluid decreased, and she went home in fair condition after a 9 months stay. Two months later she returned with edema and ascites. These symptoms yielded to treatment, but a streptococcus septicemia proved fatal 15 weeks after the second admission.

*Autopsy findings.* Body weight, 10 kgm. Heart weight, 100 grams. Kidneys weighed (horse shoe), 220 grams. The kidney was large pale greyish and gelatinous, the surface was smooth and the capsule stripped readily. The cortex was wide and yellowish. The cut surface bulged. Microscopically, there was cystic dilatation of the tubules, the lining epithelium was degenerated and contained fat and doubly refractive bodies. Some interstitial scarring and lymphocytic infiltration were present. The blood vessels were not thickened. Many glomeruli were scarred and hyaline and the tufts were often adherent to Bowman's capsule, but there were no crescents. No arteriolar sclerosis nor cardiac hypertrophy was found. In addition to the pathological changes in the kidney there was streptococcus hemolyticus septicemia with peritonitis, pleurisy, pericarditis and abscess of the cheek. There were also noted fatty degeneration of the aorta and liver, acute splenitis and parenchymatous degeneration of organs.

*Case 2 Hospital No. 5102.* Male, 3 years. His family history, birth, and development were not of significance. He suffered from a chronic cough, and on two occasions had febrile attacks considered to be tonsillitis. Edema began insidiously two months before admission. Three days before he came to the hospital, there was increase in the edema, and later vomiting, thirst, and malaise. He was febrile on admission. The heart and lungs presented no abnormality. A moderate grade of ascites and peripheral edema were present. Blood pressure was 102-64. The urine showed a specific gravity of 1031, sugar absent, albumin 16 gm. per liter (Esbach). Blood urea nitrogen was never dangerously high. In the centrifugings were many hyaline and granular casts, pus, red cells, red cell casts, and epithelial casts.

The blood count showed red blood cells, 4,510,000, white blood cells 32,000, hemoglobin, 9.1 volumes per cent oxygen capacity. After two weeks stay the edema decreased rapidly, and his progress for the next 4 weeks seemed favorable, except for a persistent leucocytosis. Then came a coryza, followed 10 days later by fever and prostration. Finally an acute colitis and peritonitis ended the picture.

*Autopsy findings.* Body weight, 16 kgm. Heart weight, 95 grams. Kidneys weighed 170 grams. The surface of the kidney was smooth, pale, and greyish yellow in color, the capsule was not adherent, the stellate veins were prominent. The cut surface bulged, the cortex was greyish yellow and well differentiated from

the medulla. The markings were fairly distinct. Microscopically there were no glomerular crescents, but the glomeruli were cellular and somewhat congested. The tubular cells were definitely large, swollen and pale and many contained fat and doubly refracting bodies. There were no vascular changes. Other pathological findings were purulent peritonitis and ulcerative colitis (*B. dysenteriae*), septicemia, sero fibrinous pleurisy and focal pneumonia (*B. coli communis*).

*Case 3 Hospital No 4546* Male, aged 14. His history included removal of adenoids and tonsils 3 years before. Symptoms began with edema a week before admission which led to the discovery of albuminuria. He presented edema of face, abdominal wall, legs, and sacral region. He was a mouth breather, with a facies of marked "adenoid" type. A little muco pus was to be seen in the nasopharynx. There was a small amount of pleural effusion. There was no radial sclerosis. The heart showed no abnormality. The blood pressure was 135-90. The urine was smoky, and contained 4 grams of albumin per liter (Esbach). In the deposit were numerous red and white cells, and a fair number of coarsely granular casts. Red blood cells, 4,150,000. White blood cells, 14,000. The Wassermann reaction was negative. The edema gradually subsided, but the hematuria persisted. The anemia grew worse, and the fundi which had been normal on admission developed papilledema, exudate, and a few hemorrhages. He was discharged after 10 months stay. He led a life of limited exertion for 8 months longer, when signs of acute illness supervened. He returned to the hospital, where he died of general septicemia. Hemolytic streptococci were isolated from the blood cultures.

*Autopsy findings* Body weight, 38.6 kgm. Heart weight, 290 grams. The kidneys weighed 430 grams. They were large, pale, slightly granular, and on section they bulged and the cortex was pale yellow and wide. Microscopically the glomeruli were large and cellular, some showed partial, others complete, hyalinization, but the majority were little affected. A few crescents were found. The tubules were slightly degenerated and some contained fat. Little scarring was noted and only slight infiltration with small round cells. No doubly refracting bodies were present. The vessels were not thickened. Other pathological lesions were *Streptococcus hemolyticus* septicemia, with peritonitis, pleurisy, pneumonia and pericarditis, fatty degeneration of the aorta and acute splenitis. No cardiac hypertrophy nor arteriolar sclerosis was present.

*Case 4 Hospital No 4421* Male, aged 21. The patient complained of blurred vision and swollen legs. His antecedents were irrelevant. Nocturia had begun 6 months before, and loss of appetite at about the same time. His vision had been blurred for a month, and for 3 weeks palpitation and dyspnea had been present. Edema of the legs was first noted 3 days before admission. His complexion was pale and sallow. One tonsil was enlarged. The chest was negative. The heart extended 4.5 cm to the right, and 10 cm to the left of the mid-line.

The radial vessels showed no sclerosis. Blood pressure 200-140. There was no edema on admission. The urine showed intense albuminuria with cellular, hyaline, and granular casts with many red and a few white cells. The Wasserman reaction was negative. The fundi showed marked neuro-retinitis, with numerous patches of exudate and superficial hemorrhage. His functional findings showed a terminal condition. He stayed 3 months in the hospital, with some symptomatic improvement. He returned 18 days after his discharge with a severe acidosis, and died 3 days later in a uremic convulsion.

*Autopsy finding:* Body weight, 63.1 kgm. Heart weight, 400 grams. Kidneys weighed 230 grams. These were contracted, granular and pale. Microscopically the glomeruli were anemic and the majority showed extensive changes, hyalinization, fibrosis or crescent formation. There was extensive interstitial fibrosis with distortion and dilatation of tubules which showed degenerating epithelium and contained casts. There were no thickened blood vessels. Fat was present in some tubules. In short, the kidneys showed extensive and active inflammatory changes but the arterioles were unaffected. Other findings besides glomerular nephritis were edema, ascites, hydro-pericardium and lobular pneumonia. No cardiac hypertrophy and no arteriolar sclerosis was present.

*Case 5 Hospital No 5014* Female, aged 27. She complained of headache, vomiting and defective sight. Her personal history included a simultaneous attack of measles and scarlet fever at 10 years, when she was very ill for some weeks. Since childhood she had had repeated sore throats, which were not relieved by a tonsillectomy at the age of 11 years. For 7 years she had had repeated quinsy. Symptoms of an earlier acute nephritis were lacking. She felt well until a month before admission, when morning nausea began, and was followed rapidly by headache, vomiting and failure of vision. For 4 weeks she neglected medical advice, and there was rapid downward progress. She seemed weary and depressed, her face was pale and a little puffy. The throat was scarred and inflamed. Cardiac dullness was increased in area. The peripheral arteries were definitely thickened. Blood pressure was 180-125. There was no edema. The fundi showed narrowed arteries, whose walls appeared thickened and tortuous. The disc margins were obscured with exudate. Stellate areas of degeneration were seen in the macular regions. The left fundus showed many small hemorrhages. Power to concentrate urea was almost nil. The urine showed albumin 2.2 grams per liter. There were hyalo-granular casts and fairly numerous red and white cells. Red blood cells 3,360,000, white blood cells, 6,800. Wassermann reaction was negative. There was no acidosis on admission but the pH and plasma CO<sub>2</sub> later fell to pathological values. Her clinical condition grew rapidly worse with corresponding laboratory findings and she died in uremia 24 days after admission.

*Autopsy findings:* Body weight, 45.7 kgm. Heart weight, 330 grams. Kidneys weighed 180 grams. They were small, the capsules stripped with difficulty, the surfaces were pale and finely nodular. On section the cortex appeared very narrow.

and the arteries stood out prominently Microscopically a considerable amount of fibrous tissue was seen replacing and distorting the tubules which were dilated, filled with fluid and lined by low epithelium They contained some fat Lymphocytes were present in the scars The glomeruli were anemic, many were scarred and surrounded by fibrous tissue, while others showed hyaline replacement. Crescents were easily found The small vessels showed very extreme and widespread fatty degeneration and thickening and some elastic tissue increase Arteriolar sclerosis was present also in the spleen, liver and pancreas Other findings were lobular pneumonia and pulmonary edema

*Case 6 Hospital No 4772* Female, aged 34 She complained of nausea, vomiting and failing vision Her father had died of Bright's disease at 40 years of age At 7 she had had scarlet fever The first symptoms began 4 months before admission Nausea, anorexia, and vomiting, asthenia and loss of weight followed Her kidney disease was then discovered, seven weeks before admission she became bed ridden, on account of weakness Her vision had failed Her blood pressure had been noted as 260 Vomiting persisted On admission her complexion was poor, the face puffy The heart was slightly enlarged The blood pressure was 230-150 The fundi showed a typical albuminuric retinitis The urine contained 7 grams of albumin per liter (Esbich) with red, white, and epithelial cells and granular casts Hemoglobin was 9.8 volume per cent oxygen capacity The Wassermann reaction was negative There was an alkalosis on admission, which was probably to be ascribed to her vomiting This gave place to a compensated acidosis during her stay There was a low intermittent fever of unexplained origin Three weeks after her admission she developed symptoms of pyelitis and cystitis, and *Staphylococcus aureus* was cultivated from the urine She died in uremia 5 days later

*Autopsy findings* Body weight, 62 kgm Heart weight, 450 gram Kidneys weighed 290 grams They were rather soft, the capsule stripped easily, the surface was dark purplish red, not granular, and showed small abscesses The cut surface bulged and was of a purplish gray color, the markings were obscured, and abscesses were seen Microscopically there was a pyelonephritis with multiple abscesses engrafted on a chronic nephritis The small vessels were thickened and fatty The glomeruli showed partial or complete hyalinization, some fibrosis and a few crescents There was some fibrosis and distortion of tubules Cardiac hypertrophy was present and the lesions of arteriolar sclerosis were found in the kidney, spleen and pancreas, but not in the liver or adrenal Other findings were staphylococcus septicemia and fibrinous pericarditis

*Case 7 Hospital No 4829* Male, aged 28 He complained of vomiting and weakness He had had typhoid fever 3 years before A year later, he began to have severe occipital headaches, recurring once a fortnight, and shortly after, nocturia became troublesome About 2 months before admission pallor and loss of weight were noted, and vomiting began A bronchitis took him to his doctor,

and albuminuria and hypertension were then discovered. Dyspnea on exertion was noted and muscular twitchings were present 3 or 4 days before admission. When admitted he was pale and ill. The heart was enlarged, the beat diffuse, the second sound reduplicated. The peripheral arteries were thickened. Blood pressure was 204-140. There was edema of the legs and over the sacrum. The fundi showed the arteries contracted, blurring of the discs, with a few hemorrhages and retinitis pigmentosa. Power to concentrate urea was reduced to 1.6, compared with normal 40-fold concentrating power. In the urine, albumin was found, 2.5 grams per liter (Esbach). The deposit contained epithelial cells, leucocytes, a few granular casts, and very few red cells. Red blood cells, 4,130,000. Hemoglobin was 13.5 volume per cent oxygen capacity. There was no acidosis. Next day uremic convulsions appeared. Pericardial friction was heard. He died two days later of respiratory failure.

*Autopsy findings* Body weight, 55 kgm. Heart weight, 530 grams. Kidneys weighed 120 grams. There was arteriosclerosis of the renal arteriae. The kidneys were finely granular and reddish white in color. The capsule was adherent, the cortex was narrow and contained small cysts. Microscopically there was thickening with fatty intimal degeneration and elastic tissue increase in the small arterioles. There was marked interstitial scarring. The tubules were dilated and lined with low epithelium. Many scarred and hyaline glomeruli and glomerular crescents were present. Arteriolar sclerosis was found in the kidney, pancreas, and spleen, but not in the heart, liver, adrenals or testicles. The heart was definitely hypertrophied. Further anatomical diagnoses ascites, hydropericardium, edema, hydrothorax, and infarcts of lung.

*Case 8 Hospital No 4847* Male, aged 27. He complained of headache, vomiting and weakness. He was overseas with the American Expeditionary Forces for 2 years. He escaped influenza and trench fever, but suffered from a flesh wound. During the last 12 months in Europe he had occasional headaches. In 1920 he was refused life insurance on account of albuminuria. Headaches became more severe 4 months before admission, and were accompanied by anorexia. During his last 6 weeks at home he failed in strength, and 3 weeks before admission vision began to fail. There was excessive thirst and drowsiness. He seemed well nourished. His face was pale and puffy. The breath was strikingly urinous. The heart did not seem enlarged. Blood pressure was 230-125. The fundi showed blurring of the discs, with many patches of exudate and some hemorrhages. There was albuminuria to the amount of 2 grams per liter, and the sediment contained many red cells, some white cells and a few hyaline and granular casts. Power to concentrate urea was reduced to 2 compared with normal 40-fold concentrating power. The blood showed red blood cells 3,168,000, white blood cells, 14,800, hemoglobin, 9.5 volumes oxygen per cent oxygen capacity. The Wassermann reaction was negative. There was a marked acidosis, which was combatted with sodium bicarbonate in five gram doses thrice daily. This treatment relieved the acidosis, and for the first 4 or 5 weeks there was some symp-

tomitic improvement. Then he sank gradually, twitchings developed and he died in uremia.

*Autopsy findings* Body weight, 65 kgms Heart weight, 490 grams Kidneys weighed 130 grams The capsule was slightly adherent, the surface was very pale and slightly granular The cut surface showed poor differentiation of the cortex and medulla, and the cortical substance was slightly narrowed, pale and gelatinous Microscopically there was very extensive scarring by dense hyaline and vascular fibrous tissue which was infiltrated with lymphocytes, the kidney substance being relatively inconspicuous Normal glomeruli were sparse, some showed crescents and endoglomerulitis with fluid in Bowman's space The majority were the seat of partial or complete hyalinization with periglomerular fibrosis, the tissue being arranged in concentric layers The tufts were avascular The tubules were cut off into islands by fibrous tissue and were dilated and lined with low epithelium They contained serous and hyaline material and cellular casts The small blood vessels showed thickening of the walls, narrowing of their lumina and fatty degeneration of the intima with lamination and increase in the elastic tissue fibres Arteriolar sclerosis was found in the pancreas Other findings were hypertrophy of the heart, ascites, and hydrothorax, pulmonary infarction, and edema

*Case 9 Hospital No 4863 Female, aged 33* She complained of shortness of breath, cough, and failing vision She had had adenoids and had suffered from sore throats in childhood She had borne four children In January, 1921, she had noted swelling of her feet, and her physician diagnosed kidney trouble Headache and palpitation followed, and caused her to spend July and August 1922, in the hospital She did well for 1 year, though the same symptoms were sometimes present In October, 1923, her dyspnea was worse and accompanied by a cough There was orthopnea and nocturia Her eyesight became impaired 7 weeks before her admission on December 1, 1923 Temperature was 102.6°F, pulse, 116, respirations, 28 She could not lie down There were signs of fluid in both chest cavities, and crepitations throughout the lung areas The area of cardiac dullness was increased and a faint systolic murmur was heard, and at the base a gallop rhythm There was a good deal of thickening of the radials The pulse was regular The abdomen showed nothing abnormal There was moderate edema of the feet, legs, and sacro lumbar region The eye-grounds revealed tortuous arteries, with exudate here and there There was no retinal hemorrhage The urine contained albumin, up to 4 grams per liter and an abundance of hyaline and granular casts Urea concentrating power was only 7-fold, compared with normal 40-fold A moderate number of white cells and a few red cells were seen Red blood cells 2,368,000, white blood cells, 8,650, hemoglobin, 7.8 volumes per cent oxygen capacity Her heart responded to treatment and she went home after 7 weeks, feeling much improved She returned on June 17, with a recurrence of cardiac decompensation, and died of heart failure with terminal pneumonia on July 11, 1924

*Autopsy findings* Body weight, 51.5 kgm. Heart weight, 555 grams Kidneys weighed 150 grams. The capsule stripped with difficulty The surfaces were pale, grayish yellow, streaked with fine vessels and finely granular The cut surface was very pale, the cortical substance narrow, the glomeruli stood out as fine points One small cyst was seen in the left kidney and a depressed infarct in the right The differentiation of cortex and medulla was obscured Microscopically there were local areas of scarring, causing puckering of the surface These scars were infiltrated with small round cells The glomeruli were anemic and many had undergone complete or partial hyaline replacement Others were surrounded by concentric layers of fibrous tissue Crescents were readily found The tubules were mostly lined with low epithelium and contained casts. They were not dilated. The small vessels were greatly thickened and contained fat in the intima. Fat was also present in some tubules. Arteriolar sclerosis was found in the pancreas, spleen, and uterus. Other findings were cardiac hypertrophy, atheroma of aorta, fluid in all serous cavities, anasarca, lobular pneumonia, brown induration of lung, and healed gastric ulcers.

*Case 10 Hospital No 4410* Male, aged 17 His history disclosed no etiology for his nephritis. The onset was marked by dyspnea, with swelling of his legs and face A few days later he went to the hospital where acute Bright's disease was diagnosed. His edema disappeared, but a recurrence with cardiac irregularity brought him to this institution There was general anasarca, and fluid in the abdominal and in both pleural cavities. Nodal rhythm was present Blood pressure 184-136 Retinoscopy showed marked papilledema, and later exudative retinitis, with slight hemorrhage. He was anemic. The urine showed much albumin, with a good many red and white cells, many hyaline and granular casts, and occasional blood casts. There was a marked uncompensated alkali deficit. Convulsions developed Improvement was slow, but he was able to go home 11 months later He returned frequently for examination, and a tendency to edema was always present, together with anemia. He died 3 years and 2 months after the onset of his symptoms.

*Autopsy findings* Body weight 66 kgm. Heart weighed 550 grams Kidneys weighed 250 grams The capsule stripped with slight difficulty exposing a pale, finely granular surface, streaked with fine vascular twigs. On section the cut surface was pale the cortex and medulla were fairly well differentiated, the cortex was narrow and pale yellow and the glomerular markings were evident. Microscopically the kidney showed extensive scarring The glomeruli were extensively involved showing partial or complete hyaline transformation and periglomerular fibrosis. Others were anemic Crescents were sparse. The tubules were degenerated in places and in others lined with low epithelium There was evidence of regeneration A few of the smaller vessels showed fatty degeneration and thickening Arteriolar sclerosis was found in the pancreas spleen, liver and pleura as well as in the kidney Other findings were cardiac hypertrophy, fatty degeneration of the heart, lobular pneumonia atherosclerosis calcareous mesenteric lymph glands, ascites hydrothorax and hydropericardium

## EXPLANATION OF FIGURES

## PLATE 1

FIG 1 CASE 9 ARTERIOLE LESION OF DIFFUSE HYPERPLASTIC SCLEROSIS IN THE KIDNEY

Frozen section stained with Scharlach R and hematoxylin Camera lucida painting  $\times 360$

FIG 2 CASE 8 ARTERIOLE LESION OF DIFFUSE HYPERPLASTIC SCLEROSIS IN THE PANCREAS

Frozen section stained with Scharlach R and hematoxylin Camera lucida painting  $\times 360$

FIG 3 CASE 5 ARTERIOLE IN THE KIDNEY

Stained by Weigert's Elastic Tissue Method Camera lucida painting  $\times 360$

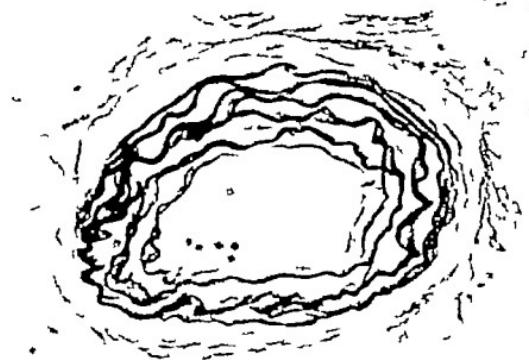
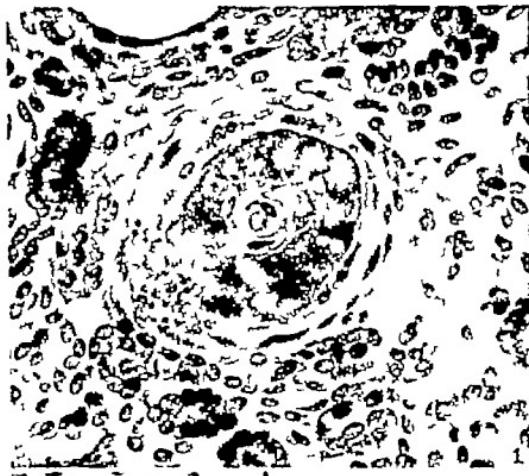
## PLATE 2

FIG 4 CASE — THREE ARTERIOLES IN THE MEDULLA OF THE KIDNEY

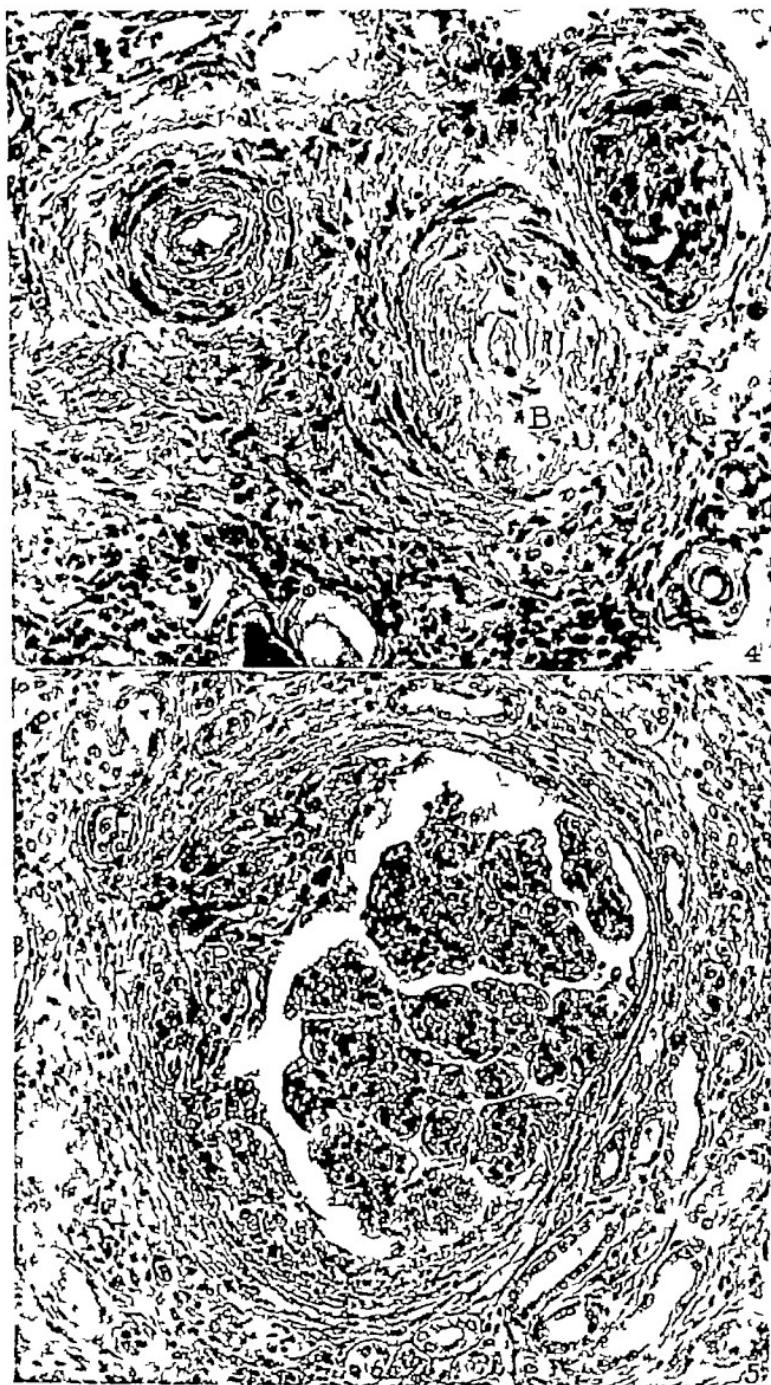
A Endothelial proliferation B and C Narrowing of lumen due to thickening of wall Microphotograph  $\times 300$

FIG 5 CASE 4 A GLOMERULUS SHOWING A TYPICAL CRESCENT (P) WHICH WE CONSIDER DIAGNOSTIC OF GLOMERULONEPHRITIS

Microphotograph  $\times 250$







(Branch and Lioder: Chronic nephritis.)



# THE EFFECT OF BREATHING OXYGEN-ENRICHED AIR DURING EXERCISE UPON PULMONARY VENTILATION AND UPON THE LACTIC ACID CONTENT OF BLOOD AND URINE

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The marked increase of pulmonary ventilation during exercise is caused primarily by the more rapid formation of carbon dioxide, which causes an increased tension of this gas in the blood and alveolar air. Douglas and Haldane (1) showed that if the exercise were not too strenuous the increase in pulmonary ventilation and the increase in alveolar carbon dioxide tension bore approximately the same relation to each other as when the ventilation was increased by the addition of carbon dioxide to the respired air. Thus, according to Haldane (2) the hyperpnea of mild or moderate exercise can be attributed solely to the increased tension of carbon dioxide. When the exercise is more strenuous, the pulmonary ventilation increases out of proportion to the increase of alveolar carbon dioxide tension. Under these circumstances some factor, or factors, additional to the carbon dioxide tension contributes to produce the hyperpnea. One of these contributory factors is the excessive formation of lactic acid in the exercising muscles. Its accumulation produces a lactic acid acidosis, and during strenuous exercise lactic acid salts escape into the blood and urine. A second cause of excessive hyperpnea during moderate and strenuous exercise is oxygen want, for Briggs (3) showed that oxygen inhalations lessen the hyperpnea, at least in individuals who are not in excellent physical condition. Since removal of the lactic acid formed during exercise is an oxidative process, the inhalation of oxygen during exercise might influence the pulmonary ventilation through lessening the accumulation of lactic acid in the muscles and in the body at large. In the following

investigation the subjects performed measured exercises and the effect of breathing oxygen-enriched air upon the lactic acid content of blood and urine was studied

*Method* Exercise was performed on a treadmill, the steps of which were 7 inches high. This treadmill was driven by an electric motor acting through a worm gear. The rate was such that approximately 85 to 90 steps were ascended per minute. This rate varied slightly from day to day. Also the rate became slightly more rapid as the exercise proceeded. In each experiment the number of steps ascended was counted each minute so that the effect of slight variations in rate could be estimated. While the rate of climbing the treadmill remained approximately constant, the amount of work performed was varied either by altering the duration of the exercise or by having the subject carry a load of 30 or 45 pounds.

During the exercise the subject breathed through a rubber mouthpiece, with the nose closed by a clip. Flutter valves directed the expired air to a series of Douglas bags. Minute collections were made, and the minute volumes later determined by passing the air from the bags through a gas meter. The intake tube was connected either with outside air or with a tank containing either pure oxygen or a mixture of outside air and oxygen (approximately 40 per cent oxygen).

Urine was collected for a period of one-half to one hour before the exercise, and for a period of approximately one hour after the exercise. Blood was drawn from the arm vein immediately before the exercise and again at about four minutes after the exercise. The concentration of lactic acid and related bodies in each specimen of blood and urine was determined by the method of Clausen (4) using permanganate oxidation as recommended by Long (5). In the case of the blood, comparisons were made between the concentrations before and after the exercise. In the case of the urine, the rate of lactic acid excretion before the exercise was determined, and the subsequent excess above the resting rate was attributed to the exercise. Blood specimens were drawn into a syringe moistened with a saturated solution of potassium fluoride. In drawing the blood the veins were temporarily obstructed, for we were unable to confirm the observation of Mendel, Engel and Goldscheider (6) that venous stasis materially alters the concentration of lactic acid in the blood drawn.

The experiments on A W H and J K L were performed in the morning after the usual breakfast, those on M S L before breakfast. No attempt was made to alter the usual daily activity of the subjects either before or after the exercise. J K L is 27 years old, weight 64.8 to 65.9 kilos without coat and vest, height 183 cm and vital capacity 4,500 cc. A W H is 50 years old, weight 70.8 to 72.3 kilos without coat and vest, height 168 cm and vital capacity 3,400 cc. M S L is 24 years old, weight 63 kilos, height 168 cm and vital capacity 4,500 cc. All the subjects were accustomed to take a moderate amount of exercise, but none was in training.

## EFFECT OF OXYGEN ON LACTIC ACID

Preliminary experiments with A. W. H. showed that in order to produce an unmistakable rise in the urinary output of lactic acid considerable exercise was necessary. Climbing the treadmill for five minutes without load or climbing for one minute with a load of 45 pounds caused no definite alteration in the lactic acid of the urine. On the other hand when thirty pounds were carried up the treadmill for five minutes at an average of 80 or more steps per minute, or when 45 pounds were carried for three minutes, there was uniformly a considerable increase in the concentration in the blood and in the urinary output of lactic acid. For this reason we first adopted the exercise of carrying 30 pounds up the treadmill for five minutes as that with which to test the effect of breathing oxygen-enriched air upon the lactic acid in the blood and urine.

The inhalation of oxygen-enriched air during this fairly strenuous exercise produced subjective sensations of less effort, less shortness of breath and less fatigue. Objectively, as Briggs (3) has shown, the volume of air breathed was less (table 1).

The increases of lactic acid in the blood and urine produced by this exercise are shown in table 1. Of the three subjects J. K. L. showed a much greater rise of lactic acid in the urine. This was probably due to his being unaccustomed to this particular exercise, for the experiment of March 18, 1925, was the first that he had performed on the treadmill, whereas A. W. H. had climbed the steps on many previous occasions. Table 1 shows how J. K. L.'s output of lactic acid in the urine decreased as he repeated the exercise. By regular exercise he subsequently reduced his lactic acid output to a low level (12). The inhalation of oxygen-enriched air during this exercise produced a definite increase in the blood level and in the urinary elimination of lactic acid.

We have stated that in preliminary experiments no definite increase in the urinary output of lactic acid could be demonstrated after walking up the treadmill for five minutes without a load. Nevertheless a slight though definite increase occurred in the blood lactic acid after this exercise (table 2). This increase was lessened by inhalation of oxygen, as were also the minute volumes of the respiration.

## EFFECT OF OXYGEN DURING EXERCISE

TABLE 1

*Effect of moderately strenuous exercise (carrying 30 pounds on treadmill for 5 minutes) on pulmonary ventilation and upon the lactic acid content of the blood and urine*

Subject	Date	Breathed	Work done kgm	Pulmonary ventilation				Lactic acid			
				Minute of exercise				Urinary excess mgm	Blood		
				Second liters	Third liters	Fourth liters	Fifth liters		Before mgm per 100 cc	After mgm per 100 cc	Increase mgm per 100 cc
	1925										
A W H	March 13	Air	6,257					75 6	47		
A W H	March 16	Air	6,439	53 1	70 9	75 9	80 5	141			
A W H	April 1	Air	6,438	51 6	65 9	77 0			51		
A W H	April 7	Air	6,362	52 0	67 5	71 6	79 3	54			
Average		Air	6,374	52 2	68 1	74 8	78 4	73			
A W H	March 17	O <sub>2</sub>	6,469	51 1	60 5	60 2			43		
A W H	March 23	O <sub>2</sub>	6,265	47 9	61 7	66 8	69 6	33			
A W H	March 31	O <sub>2</sub>	6,293	49 2	60 5	65 4			5		
A W H	April 6	O <sub>2</sub>	6,386	45 2	59 9		69 4	15			
Average		O <sub>2</sub>	6,353	48 3	60 6	64 1	69 5	24			
J K L	March 18	Air	5,802	54 6	66 6	68 9	71 3	458			
J K L	March 19	Air	5,570	49 4	58 8	63 4	68 3	329			
J K L	April 2	Air	5,949	53 4	63 8	67 4		309			
J K L	April 10	Air	5,827	46 9	55 1	65 9	65 5	199			
Average		Air	5,787	51 1	61 1	66 4	68 4	324			
J K L	March 20	O <sub>2</sub>	5,823	42 1	47 7	48 7	64 1	282			
J K L	March 24	O <sub>2</sub>	5,775	40 6	50 7	50 4	56 2	208			
J K L	April 3	O <sub>2</sub>	6,006		60 2	61 4		157			
J K L	April 8	O <sub>2</sub>	5,831	43 1	49 8	55 4	56 0	145			
Average		O <sub>2</sub>	5,859	41 9	52 1	53 9	58 8	198			
	1926			First second and third minutes							
M S L	April 9	Air	6,040	221 6	82 8	83 5	11	16	66	50	
M S L	April 12	Air	5,966	208 7	84 9	87 5	7	20	105	85	
M S L	April 14	Air	6,136	218 0	91 0	91 5	20	9	63	54	
M S L	April 21	Air	6,253	209 4	85 0		76	7	70	63	
Average		Air	6,099	214 4	85 9	87 5	28			63	

TABLE 1—Continued

Subject	Date	Breathed	Work done	Pulmonary ventilation				Lactic acid			
				Minute of exercise				Blood			
				First, Second and third minutes	Fourth	Fifth	Urinary excess	Before	After	Increase	
	1926		kgm	liters	liters	liters	mgm.	mgm. per 100 cc	mgm. per 100 cc	mgm. per 100 cc	
M S L	April 13	O <sub>2</sub>	6,229		72 5	84 6	4	10	47	37	
M S L.	April 16	O <sub>2</sub>	6 277		82 6	86 6	37	6	48	42	
M S L.	April 20	O <sub>2</sub>	6,158		74 5	81 0	16	13	60	47	
M S L	April 23	O <sub>2</sub>	6 058		69 0	76 4	12	7	53	46	
Average.'		O <sub>2</sub>	6 180		74 6	82 1	17				43
				First and second minutes	Third minute						
*M S L.	April 26	Air	4,282	147 8	87 0	166	7	88	81		
M S L	April 28	Air	4 210	135 5	99 1	200	6	78	72		
Average		Air	4,246	141 6	93 0	183					76
M S L.	April 27	O <sub>2</sub>	4,226		83 4	47	7	59	52		
M S L	April 30	O <sub>2</sub>	4 143	118 7	72 6	88	10	59	49		
Average.		O <sub>2</sub>	4,184	118 7	78 0	67					50

\* Carried 45 pounds for 3 minutes

TABLE 2

*Effect of moderate exercise (walking on treadmill for 5 minutes) on pulmonary ventilation and upon the lactic acid content of the blood and urine*

Subject	Date	Breathed	Work done	Pulmonary ventilation				Lactic acid			
				Minute of exercise				Blood			
				Second	Third	Fourth	Fifth	Urinary excess	Before	After	Increase
	1924		kgm	liters	liters	liters	liters	mgm.	mgm. per 100 cc	mgm. per 100 cc	mgm. per 100 cc
A W H	June 23	Air	5 459	47 1	60 0	60 4	65 6	0	19	46	27
A W H	June 25	Air	5 436	47 5	60 0	62 5	69 5	4	14	48	34
A W H.	June 27	Air	5 414	49 0	59 0	63 9	69 0	4	19	46	27
Average.		Air	5,436	47 9	59 7	62 3	68 0	3			29
A. W H.	June 18	O <sub>2</sub>	5,412	43 0	45 6	51 7	56 8	1	21	30	9
A W H.	June 24	O <sub>2</sub>	5 716	37 3	52 1	57 0	60 8	1	16	35	19
A. W. H.	June 26	O <sub>2</sub>	5 405	40 6	48 4	56 5	60 6	2	20	28	8
Average..		O <sub>2</sub>	5 511	40 3	48 7	55 1	59 4	1			12

## RENAL THRESHOLD FOR LACTIC ACID EXCRETION

These observations indicate that exercise may increase the blood lactic acid as determined by the Clausen method without appreciably influencing the urinary output. Inspection of our lactic acid determinations in table 2 indicates that if the lactic acid in blood specimens taken from three to five minutes after exercise was less than 30 mgm per 100 cc no excess appeared in the urine, whereas if the lactic acid in the blood exceeded 40 mgm urinary excesses appeared. Apparently then an excess of lactic acid in the urine cannot be demonstrated after exercise unless the level in the blood, by the method that we used, has been considerably increased over the normal level. The urinary output began to increase when the blood figures lay between 30 and 40 mgm of lactic acid per 100 cc of blood. We do not wish to insist upon the absolute value of these figures because the Clausen method probably determines substances other than lactic acid both in the blood and urine. But the evidence indicates that the concentration of lactic acid or related compounds in the blood may be definitely raised as a result of exercise without a demonstrable change in their excretion in the urine. It appears, therefore, that blood studies are better suited than urinary studies to show lesser changes in the lactic acid metabolism after exercise.

## EFFECT ON PULMONARY VENTILATION

The inhalation of oxygen-enriched air during the exercise that we have employed lessened the subjective sense of dyspnea during and immediately after the exercise. Objectively the minute volumes of respiration were reduced and the concentrations of carbon dioxide in the expired air were increased. These changes were observed both in the experiments which caused lactic acid excesses to appear in the urine (table 1) and in those which caused no demonstrable change in the lactic acid content of the urine (table 2). We found, then, in complete accord with the experiments of Briggs (3) that oxygen inhalations lessen the pulmonary ventilation during exercise, at least in untrained individuals. This occurs even when the exercise used does not increase the urinary output of lactic acid.

## DISCUSSION

In our experiments the inhalation of oxygen-enriched air while carrying a 30 pound load for five minutes up a treadmill lessened the subjective discomfort, reduced the pulmonary ventilation, lessened the concentration of lactic acid in the blood and diminished its output in the urine. In similar experiments without a load the respiratory effects of oxygen inhalations were similar, and the blood showed less increase when oxygen was breathed. The latter exercises, however, were not sufficient to produce a demonstrable increase in the urinary output of lactic acid even when air was breathed, probably because the blood increase was not sufficient to exceed the kidney threshold.

How do oxygen inhalations lessen the hyperpnea of muscular exercise? Douglas and Haldane (1) found that some factor or factors other than increased carbon dioxide tension contributed to the production of the hyperpnea of strenuous muscular exercise. In his discussion of these other factors Haldane (2) concluded that where an increase of lactic acid appeared in the urine this acid played a rôle in producing the hyperpnea. He pointed out, however, that in less strenuous exercise no excess of lactic acid appears in the urine and that here also the hyperpnea is lessened by oxygen inhalations. Haldane, therefore, concluded that the hyperpnea of exercise is in part due to an anoxemia which acts upon the respiratory center in a manner comparable to the anoxemia of high altitudes.

It seems probable that inhalations of oxygen produce their effect by increasing the amount and tension of oxygen in the arterial blood and by supplying more oxygen to the body. But the relief of exercise hyperpnea by oxygen is not comparable to the relief of high altitude hyperpnea by oxygen. At high altitudes there is definite arterial anoxemia, whereas Himwich and Barr (7) on man, in agreement with the animal experiments of Geppert and Zuntz (8) and Hastings (9) found no arterial anoxemia during and after vigorous exercise at sea level. On the contrary such exercise raised the oxygen saturation of arterial blood somewhat above the resting level. A fall in the oxygen saturation apparently occurs only as a result of prolonged and exhausting exercise (Harrop (10), Himwich and Barr (7)). There is then no reason to assume that an arterial anoxemia

exists during moderately strenuous exercises such as we employed Barr and Himwich (11) using exercises of approximately the same severity as ours found no fall in the oxygen saturation of arterial blood even though the lactic acid concentration in the blood was increased.

The absence of an arterial anæmia during moderately strenuous exercise does not preclude an oxygen shortage in the active tissues which may be lessened by oxygen inhalations. During exercise the utilization of oxygen by the heart and by the active voluntary muscles is extraordinarily rapid and the supply of oxygen can be maintained only by a much more rapid rate of blood flow through the active tissues. If the necessary blood flow is not maintained, an oxygen shortage might readily appear in these tissues even though the arterial blood is well saturated with oxygen. The inhalation of oxygen during exercise may increase the oxygen supply to the active muscles in two ways first by raising the oxygen saturation of arterial blood and second by enabling the heart through this better oxygen supply to maintain a more rapid circulation. Thus as a result of oxygen inhalations the active muscles may receive both a better quality and a greater quantity of blood.

The oxygen utilized by the muscles during exercise serves to remove lactic acid which has been formed during the period of contraction (A V Hill (13), Meyerhof (14)). An inadequate removal of this lactic acid leads to its accumulation in the muscles and to its escape into the blood and the urine. We have shown that the concentration of the lactic acid in the blood and the escape of lactic acid in the urine during strenuous muscular exercise are both lessened by oxygen inhalations. This lessened accumulation of lactic acid in the body would lessen the pulmonary ventilation, for the accumulation produces an acidosis which stimulates the respiratory center to increased activity. We have seen that oxygen inhalations may also lessen the pulmonary ventilation during exercises which are not sufficiently strenuous to increase the output of lactic acid in the urine. Since a certain amount of lactic acid is always formed in the muscles as a result of exercise it seems probable that the inhalation of oxygen under these circumstances influences the respiration through lessening the accumulation of acid in the muscles and the consequent change in the acid-base equilibrium of the body.

## SUMMARY

- 1 The increase in lactic acid in blood and urine resulting from measured treadmill exercises was determined
- 2 A smaller rise of blood lactic acid and a smaller excretion of lactic acid were found when oxygen-enriched air was breathed
- 3 Excess excretion of lactic acid over the resting level was only demonstrated in experiments in which the blood lactic acid rose to 30 or 40 mgm per 100 cc

## BIBLIOGRAPHY

- 1 Douglas C G , and Haldane, J S *Jour Physiol.*, 1912, **xlv**, 235 The capacity of the Air Passages under Varying Physiological Conditions
- 2 Haldane, J S *Respiration* New Haven, Yale University Press, 1922, 28-30, 156, 196 240
- 3 Briggs, H *Jour Physiol.*, 1920, **xliv**, 292 Physical Exertion, Fitness, and Breathing
- 4 Clausen, S W *Jour Biol. Chem.*, 1922 **lii**, 263 A Method for the Determination of Small Amounts of Lactic Acid.
- 5 Hill, A. V., Long C N H , and Lupton, H *Proc. Roy Soc.*, 1924 Ser B, **xvii**, 438 Muscular Exercise, Lactic Acid, and the Supply and Utilization of Oxygen
- 6 Mendel, B , Engel, W , and Goldscheider *Klin Woch.*, 1925, **iv**, 262 Über den Milchsäuregehalt des Blutes unter physiologischen und pathologischen Bedingungen
- 7 Himwich, H E , and Barr, D P *Jour Biol. Chem.*, 1923, **lvii**, 363 Studies in the Physiology of Muscular Exercise. V Oxygen Relationships in the Arterial Blood
- 8 Geppert, J , and Zuntz, N *Arch ges Physiol.*, 1888, **xli**, 189 Über die Regulation der Atmung
- 9 Hastings A B *Public Health Bull No 117*, 1921 The Physiology of Fatigue Physico-chemical Manifestations of Fatigue in the Blood.
- 10 Harrop, George A *Jour Exp Med.*, 1919, **xxx**, 241 The Oxygen and Carbon Dioxide Content of Arterial and of Venous Blood in Normal Individuals and in Patients with Anemia and Heart Disease
- 11 Barr, D P , and Himwich H E *Jour Biol. Chem.*, 1923, **lv**, 539 Studies in the Physiology of Muscular Exercise. III Development and Duration of Changes in Acid base Equilibrium
- 12 Lewis, J K., Hewlett, A W , and Barnett, G D *Proc. Soc., Exp Biol and Med.*, 1925, **xxii**, 537 The Effect of Training on Lactic Acid Excretion
- 13 Hill A V , Long, C. N H , and Lupton H *Proc. Roy Soc.*, 1924, Ser B, **xvii** 438 Muscular Exercise, Lactic Acid, and the Supply and Utilization of Oxygen
- 14 Meyerhof O *Ergebn d Physiol*, 1923, **xxii** 328 Die chemischen und energetischen Verhältnisse bei der Muskelarbeit



## CALORIMETRIC STUDIES OF THE EXTREMITIES

### I THEORY AND PRACTICE OF METHODS APPLICABLE TO SUCH INVESTIGATIONS

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#### INTRODUCTION

In 1912 Stewart delivered the Harvey lecture (9) on "Studies on the circulation in man," dealing almost entirely with blood flow in the hands and feet of both normal and abnormal persons. This lecture was preceded by two papers (8) concerning the measurement of blood flow in the hand and the effects of reflex vasomotor excitation. Since then, so far as I know, no additional experimental work on this subject has appeared in print other than the papers by Taylor.

In all of his writing Stewart adheres to the statement, "the quantity of blood in grammes flowing through the hand in the time of the experiment is given by the equation

$$Q = \frac{H}{T - T_1} \cdot \frac{1}{S}$$

where  $Q$  is the quantity of blood,  $H$  the heat given off by the blood,  $T$  the temperature of the arterial blood,  $T_1$  the temperature of the venous blood and  $S$  the specific heat of the blood" (9). I have not been able to accept the Stewart equation for the determination of blood flow, neither do I feel that such an equation can be correctly applied to the determination of " $Q$ , the quantity of blood flowing through the hand (or foot) in the period of observation" (8).<sup>1</sup> The heat taken up by the calorimeter obviously must

<sup>1</sup> A preliminary report and summary of the principal conclusions in this investigation were printed in Science vol. lxiv, pages 21-22, July 2, 1926. Further comments made by Dr Stewart are to be found in Science, pages 224-225 September 3, 1926.

be delivered to it by the immersed extremity and, if there are no losses in the calorimeter, the heat ( $H_e$ ) given out by the extremity must equal the heat ( $H_c$ ) gained by the calorimeter and its contents. The fact that any given extremity has in its tissues a certain amount of heat or is at any determined temperature is evidence that there is or has been some flow of blood in that extremity, but it is not patent that calorimetric measurements afford a means of determining the quantity or rate of flow of blood.

When a temperature gradient exists between the extremity and the bath into which it is immersed, there will be a flow or delivery of heat from the extremity to the water. This flow of heat must take place through the skin which has a conductivity  $K$  and a thickness  $D$ . Both of these factors no doubt vary considerably in different persons and practically nothing is known concerning either of them. Furthermore, the elimination of heat from the extremity is dependent not only on the rate or quantity of blood flow, but also on various conditions of the blood vessels and radiation factors, namely (a) dilated or constricted capillaries<sup>2</sup> or surface blood vessels, in which event the area of blood surface taking part in the transfer of heat will be increased or decreased and hence permit a greater or less transfer of heat, (b) the number of capillaries functioning, which is found to vary greatly in different individuals as has been demonstrated by microscopic studies of the capillaries of the skin (1, 2, 4, 6, 7), and (c) the capillary blood flow, dependent per se on the rate of blood flow in the capillaries and whether they are partially or wholly filled with blood at all times. In other words, the elimination of heat, as dependent on the blood per se, is conditioned by the total area of blood exposed in the surface capillaries and peripheral blood vessels, the number of capillaries functioning and the rate and quantity of blood flow in the capillaries. The transfer or conduction of heat from an extremity to the bath is dependent on the temperature gradient between the peripheral or surface circulation of blood and the immersion bath, this being determined by the conductivity and the thickness of the skin.

<sup>2</sup> When capillaries are spoken of, reference is made to surface blood vessels, the major portion of which are undoubtedly the capillaries.

THE EQUATION OF HEAT CONDUCTION AND THE RATE OF TRANSFER  
OF HEAT

In view of the foregoing remarks, the experiments of Brown, as well as those of Stewart, have to do primarily with the transfer or conduction of heat from one body to another and not with blood flow per se, these bodies being separated by an interface having a certain conductivity constant and temperature gradient. It seems logical to apply to these studies such mathematical equations as pertain to conduction of heat, and from the experimental data to draw conclusions relative to (a) the inherent heat capacity<sup>4</sup> or content of heat of the superficial tissues of an extremity, and (b) the rate of transfer or elimination of heat from the blood as dependent on its surface distribution and flow under the physical conditions of temperature of the bath, and so forth, set up in these experiments.

The well known equation for heat conduction (11), which may be referred to as the quantity of heat eliminated by an extremity placed in a bath of known temperature, is

$$Q = K (T_1 - T_2) \frac{A}{D} t \quad (1)$$

in which  $Q$  is the quantity of heat in calories conducted from the extremity to the water, that is, eliminated from the foot, in a given time  $t$ ,  $K$  is the conductivity constant,  $T_1$  and  $T_2$  are the temperatures respectively of the two bodies, one being at a higher temperature than the other,  $A$  is the area over which the conduction of heat from one medium to another takes place,  $D$  is the thickness and vascularity of the conducting layer, and  $t$  is the time of conduction of heat. Very approximately, then, the transfer of heat from an extremity placed in a calorimeter may be considered as shown in figure 1 *a* in which  $T_1$  represents the temperature of a liquid at a higher temperature than that of the bath at temperature  $T_2$ . The area  $A$ , thickness  $D$  and the thermal conductivity  $K$  are quantities which must be known in addition to the values of  $T_1$  and  $T_2$ , if the quantity of heat conducted in a given time  $t$  is to be calculated. In our present discussion,

<sup>4</sup> The inherent heat or thermal capacity of the foot represents the heat existing in the tissues with arterial flow checked.

however, it is to be remembered that conditions are somewhat different from those in the simple system shown in figure 1 *a* because there is a circulatory system which, from the physical standpoint, may be considered as consisting of two portions, a central one and a peripheral one (fig 1, *b* and *c*) This is not strictly true from the anatomic standpoint, but it is felt that this classification into superficial and deep circulatory portions is permissible The peripheral circulatory system lies in tissue, of a specialized nature, the main

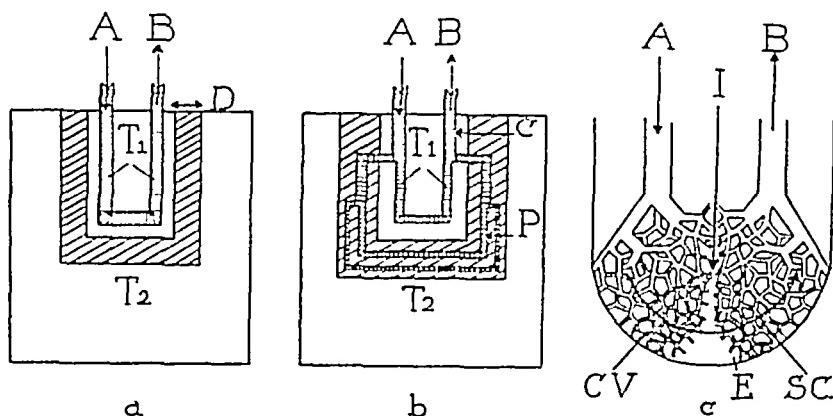


FIG 1 (a) The principles underlying the transfer of heat from a system of circulating liquid *AB* at temperature *T*<sub>1</sub> through a layer of material of thickness *D* and conductivity *K*, the temperature gradient being *T*<sub>1</sub> - *T*<sub>2</sub>, (b) the conditions when the circulatory system, from the physical standpoint, consists of two portions, a central one *C* and a peripheral one *P*, (c) diagrammatic sketch of circulation of the blood from artery *A* to vein *B* through capillary network, *C V*, capillary venules, *E*, epidermis, and *S C*, surface capillaries

function of which is the regulation of heat and thus is concerned with modifications of blood content and changes in the peripheral vessels From the physiologic side there is evidence to indicate that moderate variations in one of these two general portions of the circulatory system of an extremity may function independently of the other Recent investigations on the independent behavior of the skin capillaries when the arterial flow of blood is stopped adds some proof to this assertion (3)

It is also well known that the heat taken up by the calorimeter can be found from the equation

$$H = (m + m_w) (T_2 - T_1) \quad (2)$$

in which  $H$  is the heat in calories developed in the calorimeter, of water equivalent  $m_w$ , containing a mass of water  $m$ , while  $T_2 - T_1$  represents the rise in temperature during a given time  $t$ .

If, therefore, there are no losses in the calorimeter, or if such losses are determined and added into equation (2), then equations (1) and (2) are identities.

The temperature of the arterial blood in the extremities doubtless varies slightly in different cases. Stewart (8) carefully investigated this matter and says "We can consider that 36.7°C cannot be far from the temperature of the arterial blood in this experiment." In the investigations presented in this paper, 37°C has been taken, in general, as a satisfactory temperature, since the variation of a fraction of a degree in the value of the temperature of the arterial blood cannot affect the conclusions to be drawn.

The increase of temperature of  $M$  grams of water ( $m$  of water, and  $m_w$  the water equivalent) is

$$\frac{Q}{M} = \frac{H}{(m + m_w)} = K_1 \frac{\Delta T}{M} t \quad (3)$$

where

$$\Delta T = 37^\circ - T^\circ$$

$T$  being the temperature of the water bath and  $37^\circ$  that of the source, or

$$\frac{Q}{M} = K_1 (37^\circ - T^\circ) t$$

where

$$K_1 = \frac{K_2}{M}$$

Therefore, the *rate of increase* of the temperature of the immersion bath is given by

$$\frac{dT}{dt} = K_2 (37^\circ - T^\circ) \quad (4)$$

Integrating

$$\int \frac{d T}{37 - T} = \int K_3 dt$$

Hence,

$$-\log_{10}(37 - T) = K_3 t + C \quad (5)$$

or

$$-2.3 \log_{10}(37 - T) = K_3 t + C \quad (6)$$

And finally,

$$-\log_{10}(37 - T) + K_3 t = C_2 \quad (7)$$

in which

$$K_3 = \frac{K}{2.3 D / U}$$

It is to be noted that the term  $K_3$  includes the conductivity constant  $K$ , the area  $A$ , and the thickness  $D$

If then,  $T_1$  represents the temperature change of the extremity  $\Delta T_1$  at time  $t_1$ , and  $T_2$  the temperature change of the extremity  $\Delta T_2$  at time  $t_2$ , we have

$$\log a - \log T_1 = K_3 t_1$$

and

$$\log a - \log T_2 = K_3 t_2$$

in which  $a$  is taken as  $37^\circ\text{C}$  in my experiments

Whence (5),

$$K_3 = \frac{1}{(t_1 - t_2)} \log_{10} \frac{T_2}{T_1} \quad (8)$$

Equation (8) is, therefore, the fundamental one involved in all of these calorimetric studies, and from a determination of  $K_3$ , the rate of transfer of heat from the extremity immersed in a water bath, both in normal and abnormal subjects, it may be possible to establish conclusions of clinical importance

Objections may be raised to the statement that the temperature gradient, that is,  $\Delta T$ , is at all times equal to  $37^\circ - T^\circ$ , in which  $37^\circ\text{C}$  is taken as the temperature of the arterial blood and  $T^\circ$  is the temperature of the bath at any specified time,  $t$ . The assumption of a constant blood temperature of  $37^\circ\text{C}$  gives, in the data and curves presented in this paper, the minimal rates of heat elimination, and postulates that the temperature of the venous blood leaving the extremity is close to the temperature of the arterial blood. Stewart (8) says, "When a part is immersed for a considerable time in water of a given temperature, much the greater part of the venous blood must have a temperature not very different from that of the bath." If, therefore, we accept Stewart's conclusion, we should take as the temperature of the blood the average of the arterial ( $x^\circ$ ) and venous, or calorimetric ( $y^\circ$ ), temperatures, or  $\frac{x^\circ + y^\circ}{2}$ . Under these assumptions, therefore,

$$\Delta T = \frac{x^\circ + y^\circ}{2} - T^\circ$$

and equation (8) comes out in identically the same form and manner as on the assumption that  $\Delta T = 37^\circ\text{C} - T^\circ$ . The initial temperature level only differs in the two assumptions. Such differences in assumptions relative to the temperature of the blood, however, will give different numerical values when the experimental data are substituted in equation (8). But the ratios of the rates of elimination of heat between normal and pathologic conditions will remain the same irrespective of the temperature which is taken to represent that of the blood. For example, the value of  $K_1$  in a given normal person (curve 5, figs 3 and 6), on the assumption of a blood temperature of  $37^\circ\text{C}$ , is 0.00060, and the value of  $K_1$  in a given case of polycythemia (curve 1, figs 4 and 7) is 0.00118. The ratio of these values is practically 1.2. Taking the experimental data for the normal selected (table 1, curve 5, figs 3 and 6), and starting at the time  $t$ , which equals twenty minutes (at which time the so-called "equilibrium" conditions may be said to exist), we find that  $x^\circ = 36.7^\circ\text{C}$  and  $y^\circ = 20.7^\circ\text{C}$ , with an average value of  $29.26^\circ\text{C}$ . The calorimetric temperature is  $21.85^\circ\text{C}$  at the time  $t$ , which equals twenty

minutes Therefore, at the time,  $t = 20$  minutes,  $\Delta T = 29.26^\circ$  At the time,  $t = 80$  minutes,  $\Delta T = 30.40^\circ - 24.05^\circ$  From these data  $K_3 = 0.00112$  In a similar manner it can be shown that in the selected case of polycythemia (curve 1, figs 4 and 7)  $K_3 = 0.00231$  The ratio of these values is practically 1.2 These and other like calculations indicate that the matter of the assumption of the temperature of the blood does not enter into the discussion provided the numerical results obtained are taken as comparative and not absolute values, as is the case in these experiments The conclusions reached in this paper, therefore, are not affected by any assumptions as to the temperature of the blood, provided it can be stated that the physical and physiologic status of affairs is such that there are no radical changes in the circulatory conditions during the experimental test We are interested fundamentally in a comparison (or ratio) of rates of elimination of heat in various normal and pathologic conditions, and have no concern with the rate of flow of the blood

I desire to comment further on the importance of equation (4),

$$\frac{dT}{dt} = K (T_1 - T_2)$$

for this equation states that the rate at which a body loses heat is proportional to the difference between its temperature and that of its surroundings The rate at which an immersed extremity loses heat is the same as the rate at which heat is being gained by the calorimeter As time goes on, the temperature gradient between immersed extremity and bath decreases, there is a lessened transfer of heat and as a result the curve representing the relation between temperature and time approaches a temperature the limiting value of which is theoretically the temperature of the arterial blood

Due regard for the statements made in the preceding paragraph was not taken, I believe, by Stewart in his investigations The data on Stewart's experiment no 1, which is presumably typical of his experimental results, is quoted from one of his papers (8)

"At 1.41 p m put hands (of M C) in bath at  $27.0^\circ$  Put 3,200 cc of water in calorimeter  $L$  (left hand) and 3,000 cc in  $R$  (right hand) Room temperature  $18.8^\circ$  Mouth temperature  $36.8^\circ$  Pulse 107

Volume of right hand in calorimeter 435 cc, of left, 410 cc"

Then follow the experimental data having to do with the rise in the temperature of the calorimeter bath. The increases of temperature with time as shown by Stewart are plotted as curve 1, figure 2, the symbol (X) being for the right hand and (●) for the left one. Stewart's concluding remarks are "For the first part of the experiment (7 minutes) the flow comes out at 12.8 grams blood per 100 cc of hand per minute for the right hand, 13.9 grams for the left. For the second part (the last 7 minutes) 10.29 grams for the right and 11.73 grams for the left."

The blood flow is said to have changed from 12.8 grams to 10.29 grams in twenty two minutes or a change of  $2\frac{5}{11}6$  (average), which is approximately 20 per cent for the right hand, and for the left hand  $2\frac{17}{12}8$  (average), or about 17 per cent. That is to say, the blood flow is said to have changed about 20 per cent in twenty-two minutes in a bath at an initial temperature of  $26.75^{\circ}\text{C}$  and final temperature of  $28.78^{\circ}\text{C}$ . Such conclusions I believe to be erroneous because the relationship between rise of temperature in the calorimeter with time of immersion of an extremity, as plotted in curve 1, figure 2 (data being taken from the experiment no 1 of Stewart, to which reference has been made), is not a linear one. This absence of a linear relationship between the rise of temperature and the time, which is again emphasized by curve 2, figure 2, arises for the simple reason that the temperature gradient has changed from  $10.05^{\circ}\text{C}$  (that is  $36.8^{\circ}$  to  $26.75^{\circ}\text{C}$ ) at the beginning of the experiment, to  $8^{\circ}\text{C}$  (that is  $36.8^{\circ}$  to  $28.78^{\circ}\text{C}$ ) at the conclusion of the experiment. In these statements,  $36.8^{\circ}\text{C}$  is taken from Stewart's data as representative of the temperature of the arterial blood in an extremity. It is apparent that the difference in temperature gradient between the initial and final readings is  $2.05^{\circ}\text{C}$  or roughly a change of 22 per cent, as is seen by dividing the temperature difference of  $2.05^{\circ}$  by 9 (the average of 10.05 and 8). It is possible that these changes in temperature gradient have been incorrectly interpreted by Stewart as changes in blood flow. It is obviously a question of rates of increase of temperature of the immersion bath as given by the equation (4),

$$\frac{dT}{dt} = K_1 (37^{\circ} - T)$$

and that these increases of temperature are dependent on the rates of elimination of heat from the extremity. In the last analysis I believe that one is justified in saying that calorimetric determinations, *per se*, give data only on the intake or output of heat and not on the mechanism of the production or elimination of heat.

There is, furthermore, no evidence of any change in the rate of heat elimination and, therefore, presumably no proof of any change in the rate of blood flow, as is shown in curve 3, figure 2, in which  $\log \Delta T$ , or  $\log (36.8^\circ - T^\circ)$  is plotted against  $t$ , the time. This is very nearly a single straight line with, however, some indication of

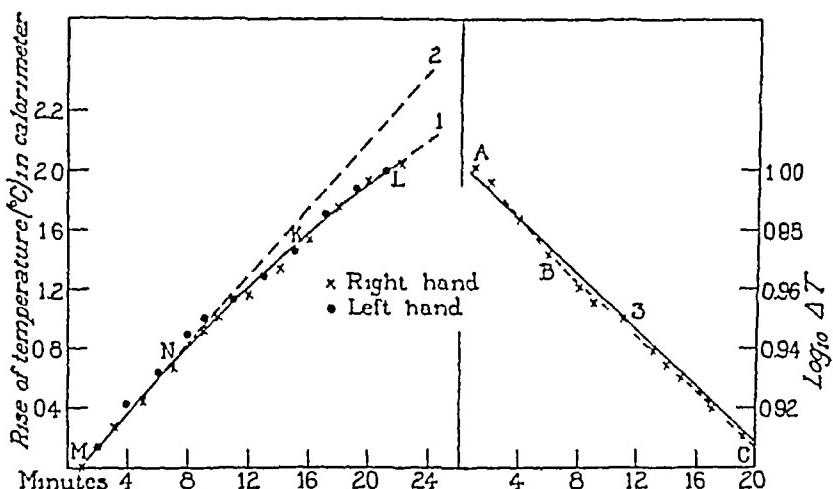


FIG 2 Curve 1, data plotted from Stewart's paper (8), curve 2, to emphasize the fact that curve 1 is not a straight line, and curve 3, Stewart's data plotted logarithmically against the time  $t$ .

two portions, marked  $AB$  and  $BC$ , respectively. The slight difference in the slope of the line during the first six minutes of immersion is doubtless due to vasomotor reactions. The curve as a whole, however, conclusively shows that the rate of elimination of heat from an extremity, as determined from calorimetric data given by Stewart, is a constant. There is, therefore, every reason to believe that the rate of blood flow remains constant, provided we include in the definition of the expression "blood flow" the various factors which are known to affect it or to be the equivalent of such flow and which are outlined in the introductory paragraphs of this paper.

## CALORIMETRIC DATA ON THE EXTREMITIES

Using the apparatus described by Kegerreis, together with accessory means of determining the area of an extremity, Brown has adopted a method of obtaining comparative heat transfers in calories for each square inch of surface. It has been his purpose to simplify the experiments clinically and he has therefore assumed a linear relationship between the rise in temperature in the calorimeter and the time after the break or knee in the curve has been passed. If his procedure is to be accepted as sufficiently exact, it must be justified by a comparison of ratios of the heat capacities of the extremities and the rates of transfer or elimination of heat due to the surface circulation as determined by the use of the logarithmic equation (8),

$$K_s = \frac{1}{t_1 - t_2} \log_{10} \frac{T_2}{T_1}$$

I believe that Brown's results are not to be accepted as specific values of the heat elimination for the particular cases cited or discussed but that, from the clinical standpoint, his simplified method, which assumes a linear relationship between the rise in temperature and the time after the so called state of equilibrium has been reached, is doubtless sufficiently accurate to permit of some segregation or groupings of cases, and gives ratios, when compared with the values he obtains for normal subjects, which are in excellent agreement with the data and ratios obtained from the methods described and discussed here. Taking the data given in table 3, and using ratios obtained from the four methods of analysis of data, I conclude that, in large part, the clinical procedures of Brown furnish data regarding the rate of transfer or elimination of heat to the calorimeter due to the superficial or peripheral circulation as conditioned by skin factors.

The curves of figures 3, 4 and 5 represent calorimetric rises in temperature Centigrade when plotted against time in minutes. Figure 3 contains curves showing the increase of temperature of the calorimeter and contents with the time of immersion of an extremity for normal persons, figure 4 for persons clinically grouped as having polycythemia, while the curves of figure 5 contain data on persons having thrombo-angitis obliterans, or Buerger's disease. These data as graphically presented are taken at random by the writer.

from the clinical experiments of Brown. It is presumed that these and other curves which will be presented will serve as illustrative materials only and that there is not to be read into these discussions any conclusions of clinical significance, for the reason that the data presented for the various clinical subdivisions selected are too meager to warrant their acceptance on any basis other than that of being

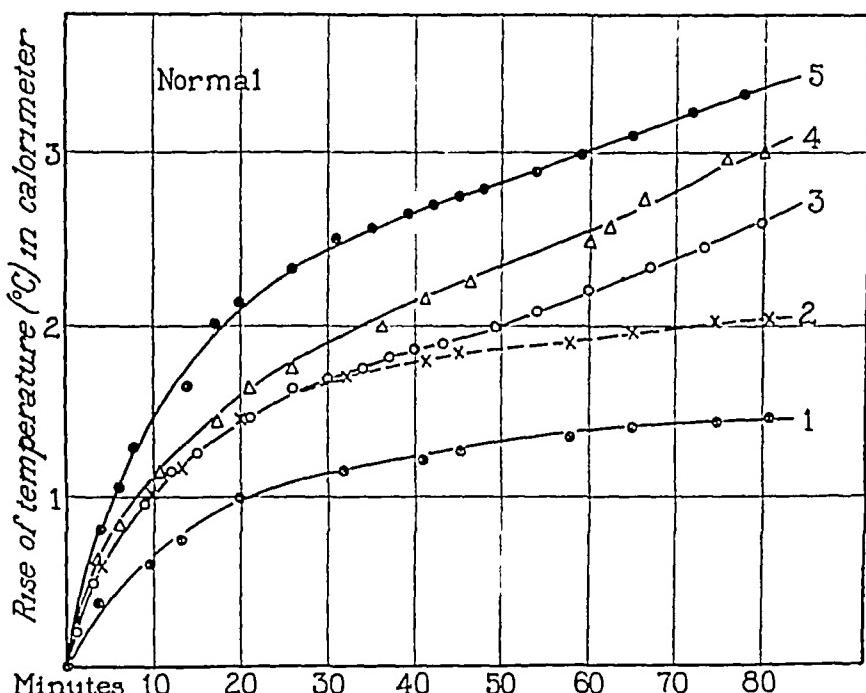


FIG. 3 CURVES SHOWING THE RELATIONSHIPS BETWEEN THE RISE OF TEMPERATURE IN THE CALORIMETER AND CONTENTS AND THE TIME (IN MINUTES) OF THE TEST

These curves represent data obtained on the same normal individual on different days

fairly representative of the various groups which are indicated clinically

In figures 6, 7 and 8 are plotted the results of the same experimental tests as are found in figures 3, 4 and 5, in terms of equation (7), that is,

$$-\log_{10} (37^\circ - T^\circ) + K_3 t = C_3$$

in which the  $v$  axis is taken as  $\log_{10}\Delta T$  and the  $x$  axis as the time ( $t$ ) in minutes. The same order of presentation of clinical classification is made as in figures 3, 4 and 5, figure 6 is for the normal, figure 7 for cases of polycythemia, and figure 8 for cases of thrombo angitis obliterans. In each instance the curve number appearing in figure 3 is carried over for purposes of identification to the results plotted in figure 6, and so on.

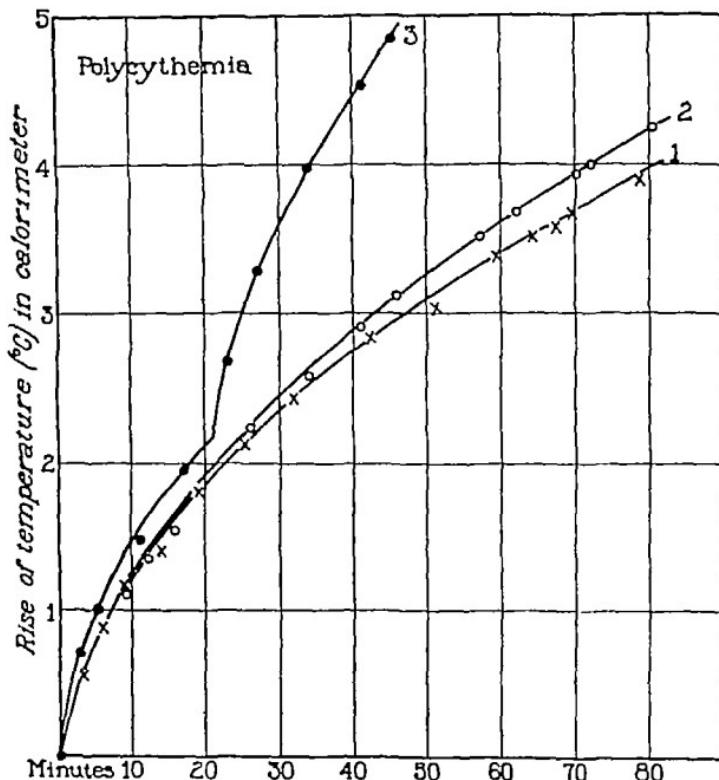


FIG 4 CURVES SHOWING THE RELATIONSHIPS BETWEEN THE RISE OF TEMPERATURE IN THE CALORIMETER AND CONTENTS, AND THE TIME OF THE TEST IN 3 CASES OF POLYCYTHEMIA

obliterans. In each instance the curve number appearing in figure 3 is carried over for purposes of identification to the results plotted in figure 6, and so on.

In table 1 is included a sample set of data for curve 5, figure 3,

as well as the values of  $\log_{10}\Delta T$  for which the corresponding curve 5, figure 6, is obtained

It is to be noted that in both the theoretic discussion and in the calculations involving equation (8) as plotted in figures 6, 7 and 8,

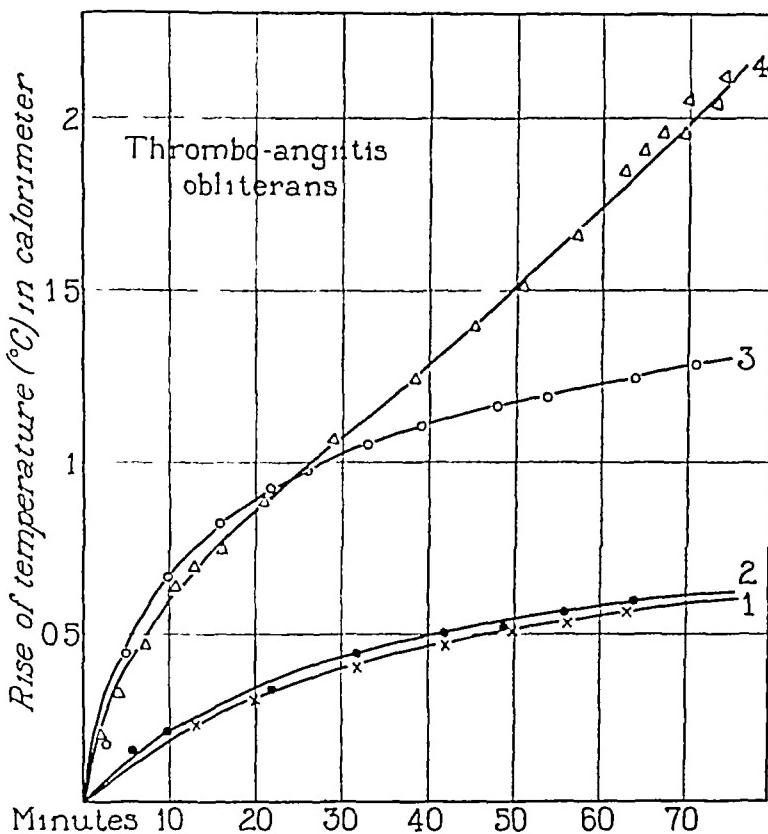


FIG 5 CURVES SHOWING THE RELATIONSHIPS BETWEEN THE RISE OF TEMPERATURE IN THE CALORIMETER AND CONTENTS, AND THE TIME OF THE TESTS IN 4 CASES OF THROMBO-ANGIITIS OBLITERANS

all reference to the effects of loss of heat by radiation from the calorimeter has been omitted. A series of cooling curves is shown in figure 9. It will be appreciated that, in general, no error comparable to other likely sources of discrepancy and irregularity is introduced by reason of this omission.

All of the curves plotted in figures 6, 7 and 8, indicating the relationship between  $\log_{10}\Delta T$  and  $t$  show two portions with decidedly different slopes, therefore indicating two distinct values of the rate of transference or elimination of heat. In the cases of normal subjects, the intersection of these two portions of these curves occurs at the point *B* (fig. 6) in from fourteen to twenty-two minutes, in

TABLE I  
Data for curve 5, figure 3, and curve 5, figure 6, normal control\*

Time	Time minutes	Beckmann reading	$\Delta T = 36.7 - T$	$\log_{10}\Delta T$
10 21	0	1 15	15 99	1 2038
10 26	5	2 03	15 12	1 1796
10 27	6	2 23	14 92	1 1738
10 29	8	2 54	14 70	1 1673
10 35	14	2 99	14 16	1 1511
10 38	17	3 17	13 98	1 1455
10 41	20	3 30	13 85	1 1414
10 47	26	3 50	13 65	1 1351
10 52	31	3 67	13 48	1 1297
10 56	35	3 73	13 42	1 1274
11 00	39	3 80	13 35	1 1255
11 03	42	3 85	13 30	1 1239
11 06	45	3 90	13 25	1 1222
11 09	48	3 95	13 20	1 1206
11 15	54	4 06	13 09	1 1169
11 20	59	4 14	13 01	1 1143
11 26	65	4 25	12 90	1 1106
11 33	72	4 38	12 76	1 1059
11 39	78	4 49	12 65	1 1021

\* August 5, 1924. Systolic blood pressure 110 and diastolic 80 pulse 70 Room temperature 25.4°C Main scale of certified Beckmann thermometer set at 19.56°C Differential values for preliminary readings were 1 13° at 10 17 a.m., 1 14° at 10 19 a.m. and 1 15° at 10.21 a.m. Arterial blood temperature taken as 36.7°C

cases of polycythemia, at not less than twenty minutes in any instance (fig. 7), and in conditions of thrombo angitis obliterans at from six to ten minutes. Two such separate or distinct portions to each curve would be expected, for during the early period of immersion there is a transfer of heat from the extremity, per se, by reason of the inherent heat or thermal capacity of the foot, plus a transference of heat due to peripheral or surface circulation, while

secondly, after that which may be spoken of as the steady state, following Stewart's general descriptive notions, has been reached, then the heat which is eliminated and transferred to the calorimeter is due to surface circulatory conditions only ( $K_s = \frac{KA}{23DM}$  of equation 7)

Neither is it to be expected that the transfer of heat due to the inherent heat capacity of the extremity plus that due to the surface

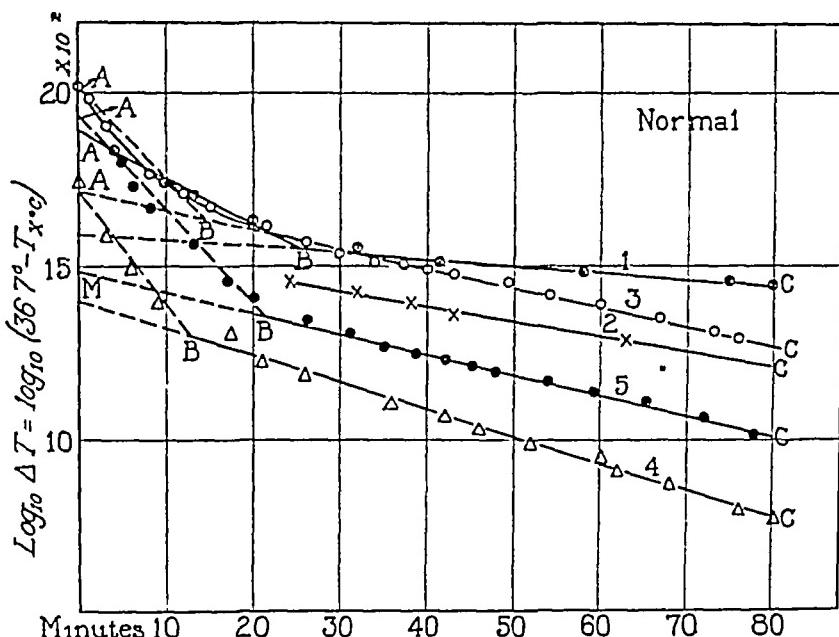


FIG 6 CURVES SHOWING THE RELATIONSHIPS BETWEEN  $\log_{10} \Delta T$  AND THE TIME  $t$  FOR A SELECTED NORMAL SUBJECT  
(See figure 3)

circulation, when plotted logarithmically against time, would give a straight line because of (a) vasomotor reactions, (b) loss of heat from the foot per se, or, as it may be called, depletion of tissue heat or heat capacity of the extremity, and (c) change or decrease in temperature gradient in the superficial tissues of the extremity with time of immersion in the bath. An inspection of the curves shows that the vasomotor reactions are largely completed in from three to six

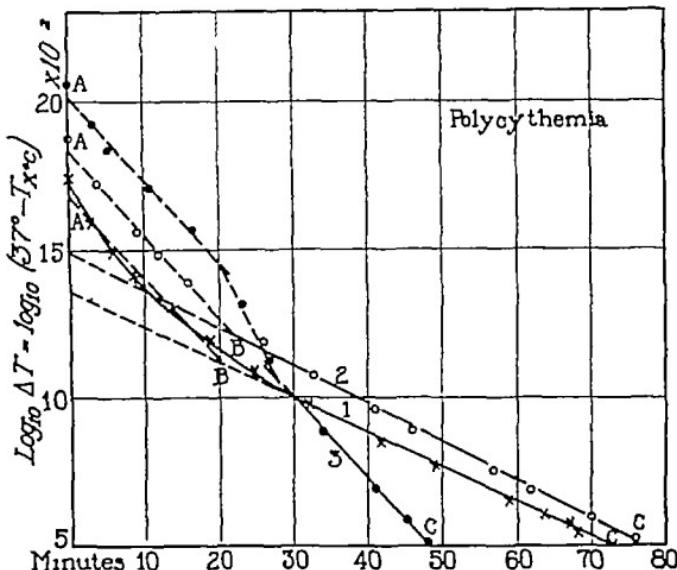


FIG 7 CURVES SHOWING THE RELATIONSHIPS BETWEEN  $\log_{10} \Delta T$  AND THE TIME  $t$  IN 3 CASES OF POLYCYTHEMIA

(See figure 4)

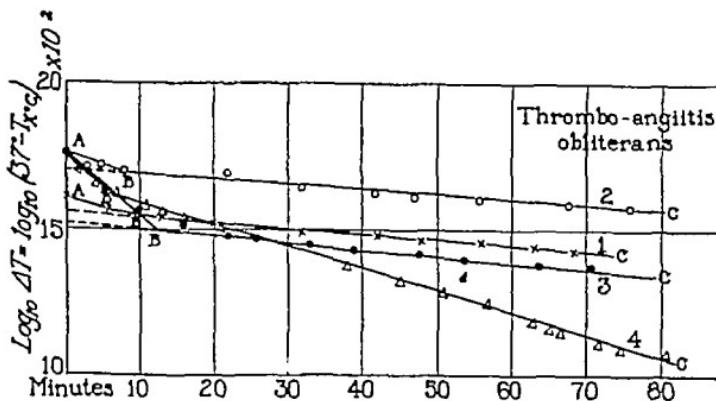


FIG 8 CURVES SHOWING THE RELATIONSHIPS BETWEEN  $\log_{10} \Delta T$  AND THE TIME  $t$  IN 4 CASES OF THROMBO-ANGIITIS OBLITERANS

(See figure 5)

minutes. This is also evidenced in the sample set of data from Stewart and is shown in curve 3, figure 2. In all the curves of figures 6, 7 and 8, it is found that, from the twenty-minute period on, there is no question that the rate of transfer of heat from the foot due to the superficial or peripheral circulation or distribution of the blood remains constant for each particular case. Hence the relationship

$$K_3 = \frac{1}{t_1 - t_2} \log_{10} \frac{T_2}{T_1}$$

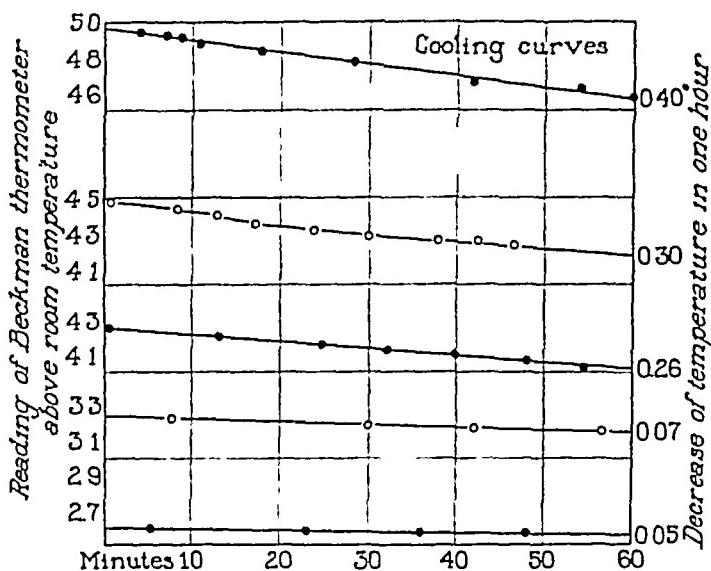


FIG 9 CALORIMETRIC COOLING CURVES

is proved applicable to these investigations. The value of  $K_3$  (on the assumption that  $\Delta T = 37^\circ - T^\circ$ ) for each of the curves of figures 6, 7 and 8 is given in table 2.

It is possible, therefore, to analyze all of the curves of figures 3, 4 and 5 into two portions, namely, the heat delivered to the calorimeter by means of the inherent heat or thermal capacity of the extremity proper, and the heat given up to the calorimeter as a summation effect due to the peripheral blood vessels or surface circulation. Figures 10, 11 and 12 show this analysis graphically. There are three curves in each of these figures, curve 1 represents the heat

capacity or inherent heat of the superficial portions of the extremity plus the elimination of heat due to the surface circulatory system, curve 2, the heat eliminated by reason of the surface circulation only, and curve 3, which is the difference between the readings

TABLE 2

$$\text{Values of } K_2 \text{ obtained from the equation } K_2 = \frac{1}{t_1 - t_2} \log_{10} \frac{T_2}{T_1}$$

Figures	Curves	Clinical classification (Brown's data)	$\frac{T_2}{T_1}$	$K_2 = \frac{1}{t_1 - t_2}$	$K_3$ for each square inch each minute	Average $K_2$
3, 6	1	Normal (same person and different conditions of summer weather)	0 00020*	17 $\times 10^{-7}$		
	2		0 00045	39		
	3		0 00053	46		
	4		0 00073	63		
	5		0 00060	52		52 $\times 10^{-7}$
		(Another person)	0 00076	62		
4, 7	1	Polycythemia	0 00118	104 $\times 10^{-7}$		
	2	Polycythemia	0 00136	123		
	3	Polycythemia	0 00300*	232		(See Curve 3 figure 4)
		Polycythemia	0 00109	98		
		Polycythemia	0 00127	113		109 $\times 10^{-7}$
5, 8	1	Thrombo-angiitis obliterans	0 00020	23 $\times 10^{-7}$		
	2	Thrombo-angiitis obliterans	0 00020	23		
	3	Thrombo-angiitis obliterans	0 00022	19		
	4	Thrombo-angiitis obliterans	0 00078*	78		(Suspected Buerger's disease)
		Thrombo-angiitis obliterans	0 00019	17		
		Thrombo-angiitis obliterans	0 00017	16		19 $\times 10^{-7}$

\* These readings are excluded from the averages.

of curves 1 and 2, the inherent thermal capacity of the extremity. Figure 10 is for an average normal (curve 5, fig 3), figure 11 for a case of polycythemia (curve 1, fig 4), and figure 12 for a case of thrombo-angiitis obliterans or Buerger's disease (curve 1, fig 5). The data

for curve 2 in each of the figures 10, 11 and 12 were obtained by projecting back to intersection with the  $y$  axis ( $y = \log_{10} \Delta T$ ) the portions  $BC$  of the corresponding curves of figures 6, 7 and 8. Since the  $BC$  portions of the curves of figures 6, 7 and 8 represent the rate of transfer of heat set up by the surface circulatory system only, the values of  $\Delta T$  obtained for the initial periods of immersion from the points of the curves under consideration in figures 6, 7 and 8 give a measure

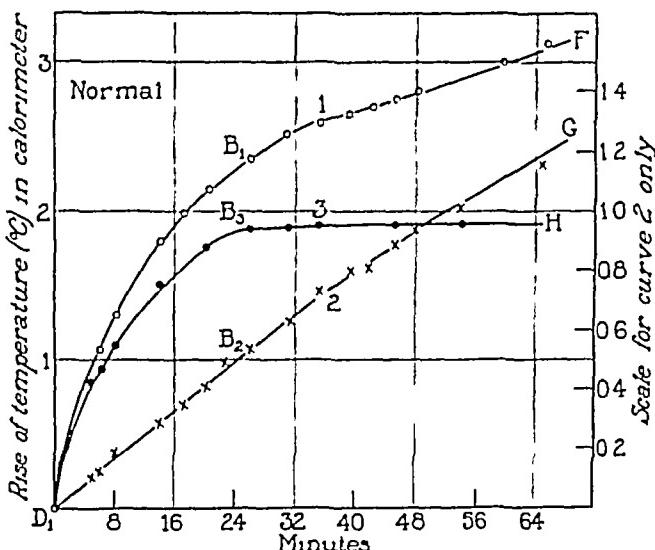


FIG 10 CURVES SHOWING THE ANALYSIS OF THE HEAT ELIMINATED FROM THE FOOT OF A NORMAL SUBJECT

Curve 1, total elimination of heat, curve 2, heat eliminated by reason of the surface circulation only, and curve 3, the inherent thermal capacity of the extremity

of the elimination of heat due to the peripheral or cutaneous circulation only. For instance, curve 2 of figure 10 is obtained by projecting the line  $BC$  of curve 5, figure 6, back to the  $y$  axis. By means of the original curve 5 of figure 3 (which is replotted as curve 1 of figure 10), the values of  $\Delta T$  for the immersion periods (in minutes) plotted in curve 1, figure 10, can be determined from the projection of the line  $BC$  (curve 5, fig 6). Obviously there is no need to proceed further than the points marked  $B$ , of figures 6, 7 and 8, since

the portions  $BC$  represent graphically the transfer of heat due to surface circulation only. To amplify these statements I have in figure 10 drawn the complete original curve  $D_1B_1F$  (curve 5, fig. 3), the corresponding complete curve  $D_1B_2G$  of heat transfer due to the temperature gradient established by surface circulatory conditions (obtained from curve 5, fig. 6) and the curve  $D_1B_3H$ , which is the difference between curves 1 and 2 of figure 10 and which therefore,

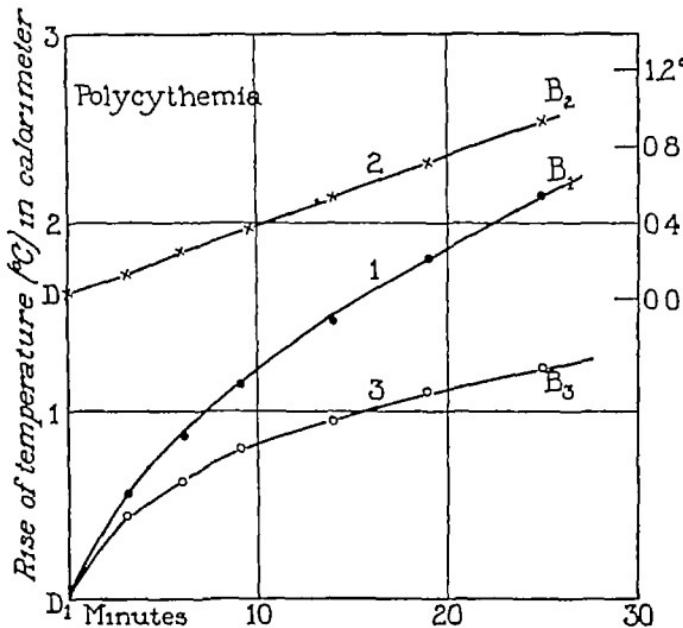


FIG. 11 SAME AS FIGURE 10 CASES OF POLYCYTHEMIA

I believe, represents the "temperature rise with time" conditions due to transfer of heat to the calorimeter from the extremity only. It will be noted that the portion  $B_3H$  of curve 3, figure 10, is a straight line parallel to the  $x$  axis, as it should be.

The data for curve 1 of figure 10 are mass of water plus the water equivalent of the calorimeter equalled 4,180 grams,  $\Delta T$  for the extremity only at the end of twenty six minutes (point  $B_1$ , curve 3) was  $1.88^\circ$ , the volume displaced by the foot immersed was 1,175 cc.,

and the area, as obtained by the Kegerreis method, was 115 square inches. These data show that the heat delivered to the calorimeter, due to the inherent heat or heat capacity of the foot, averages 0.256 calorie for each cubic centimeter each minute, or that 2.62 calories for each square inch each minute are delivered on account of this heat capacity. Likewise, considering the same period of time, it can be shown that the peripheral or surface circulation, per se, produced 0.073 calorie for each cubic centimeter each minute, or 0.75 calorie for each square inch each minute. Similar data can be obtained from the curves of figures 11 and 12, for which the accessory data are volume 1,125 cc., area 118 square inches (fig. 11), and volume 725 cc. and area 87 square inches (fig. 12).

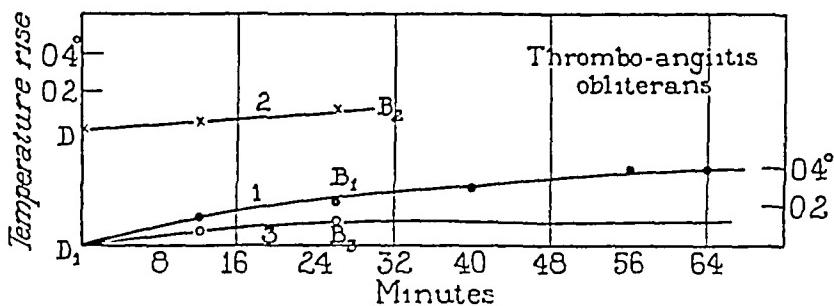


FIG. 12 SAME AS FIGURES 10 AND 11, CASES OF THROMBO-ANGIITIS OBLITERANS

Possibly an easier and just as satisfactory a method, especially in finding ratios between the inherent heats (or thermal capacities) of the extremities of normals and those obtained in cases of disease is to use a planimeter and to find the areas included between the portions  $AB$  of the curves of figures 6, 7 and 8 and the projections of the lines  $BC$  to the points of intersection at the  $y$  axis. Such an area is represented as  $ABM$  in curve 5, figure 6.

I have included in the data of table 3, under method 3, the heat capacities of the extremities as determined by obtaining the areas of  $(\log \Delta T)$  ( $i$ ). It will be noted from table 3 that the ratio of the average heat capacity of the normal by method 2 to the average normal heat capacity by method 3 is 1.91/1.52, or 1.24, and that the ratio of the average heat capacity in polycythemia by method 2 to that by

method 3 is 2 17/1 80, or 1 21. These ratios are in excellent agreement. However, in the case of Buerger's disease, this ratio as determined from a comparison of methods 2 and 3 turns out to be 0 55/0 11, or 5. Possibly no comment is necessary other than that different methods of handling data may give results which are not in themselves necessarily comparable.

TABLE 3  
*Tabulation of data for curves of figures 3 to 8 obtained by four methods*

Figure	Curve	Clinical classification	Room temperature degrees C.	Method			
				1 $K_1 \text{ from equation } K_1 = \frac{1}{1 - t_1} \log_{10} \frac{T_2}{T_1}$	2	3	4 Caories for each square inch, each minute (Brown's clinical method)
3 6	1	Normal	18	$17 \times 10^{-7}$	1 52	1 75	
	2		20	39	1 20	0 95	
	3		21	46	1 68	1 05	
	4		23	63	2 12	1 92	0 97
	5		25	52	2 62	1 90	1 03
Average				$43 \times 10^{-7}$	1 91	1 52	1 00
4, 7	1	Polycythemia	19	$104 \times 10^{-7}$	1 90	1 70	
	2		19	123	2 03	1 95	1 72
	3		20	232*	2 57	1 75	3 29*
Average				$114 \times 10^{-7}$	2 17	1 80	
5, 8	1	Thrombo-angiitis obliterans	19	$23 \times 10^{-7}$	0 49	0 10	0 25
	2		19	23	0 51	0 12	0 23
	3		25	20	1 56	0 90	0 30
	4		18	78*	0 66	0 10	
Average				$22 \times 10^{-7}$	0 55	0 11	0 26

\* These readings are excluded from the averages (see table 2)

#### DISCUSSION

The data given in tables 3, 4 and 5 enable us to draw some important physiologic conclusions. In the first place, the same person, who was clinically passed as a "normal," was subjected to a series

of calorimetric tests during the month of August, 1924. In that month the customary changes in summer weather occurred, making it possible to study somewhat the effects of out-of-door or environmental temperature on the data obtained calorimetrically. An inspection of the data in table 3, methods 1 and 2, shows an increased rate of elimination of heat due to surface circulation and also an increased inherent heat capacity with rise of environmental tempera-

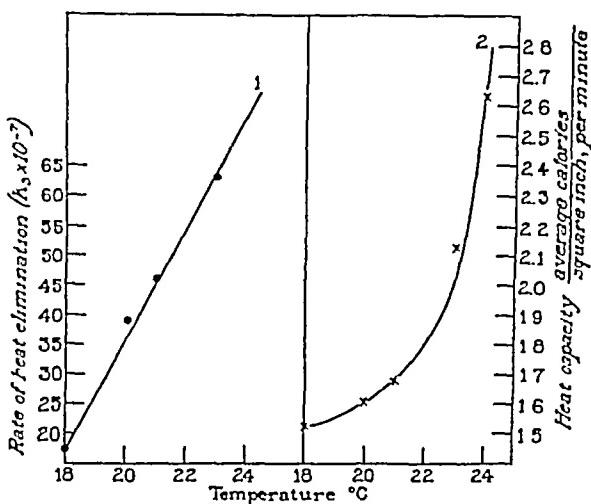


FIG 13 Curve 1, the relationship between the environmental temperature and the rate of heat elimination  $K_3$  in the case of a selected normal subject, curve 2, the relationship between the environmental temperature and the inherent heat capacity in the case of the same normal individual

tures. Figure 13 contains curve 1, in which the rate of transfer of heat to the calorimeter due to surface circulatory conditions

$$K_3 = \frac{1}{t_1 - t_0} \log_{10} \frac{T_2}{T_1}$$

is plotted against out-of-door temperatures, and curve 2, in which out-of-door temperatures and inherent heat capacities (average calories for each square inch each minute) are compared, these temperatures being practically those of the room in which the experiments were conducted. These curves are mathematically expressed

as  $y = mx + b$  (curve 1) or the equation of a straight line, and  $x^2 = 4 ay$  (curve 2) or the equation of a parabola. The values of the constant  $a$  for the various experimental values of  $x$  and  $y$  are shown in table 4.

These and similar curves and data give us a basis for certain deductions which, while they may be but a first approximation to the truth and may need further revision and amplification, are of physiologic importance. In the first place, it appears that, in normal subjects, the rate of transfer or elimination of heat due to surface circulation, that is,

$$K_1 = \frac{1}{t_1 - t_2} \log_{10} \frac{T_2}{T_1}$$

TABLE 4  
*Relationships between environmental temperatures and inherent heat capacities*

$x$	$y$	*
Temperature degrees C.	Heat capacity calories	From equation $x^2 = 4 ay$
18	1.52	54
20	1.60	62
21	1.68	65
23	2.12	62
24	2.62	56

\* For each square inch each minute.

is directly proportional to the temperature of the surrounding environment in degrees Centigrade. Secondly, the inherent heat or thermal capacity of the extremity, that is, the ratio of average calories to each square inch, each minute, increases proportionately to the square of the temperature of the environment, expressed in degrees Centigrade, and that there is for each and every normal individual a certain inherent constant  $a$ , obtained by dividing the square of the temperature in degrees Centigrade by four times the experimentally determined thermal capacity. And again, if curve 1 of figure 13 is projected back to the ordinate,  $y = 0$ , it is shown that in the case of the normal investigated, when the temperature of the surrounding medium reaches from  $15^\circ$  to  $16^\circ\text{C}$ , the rate of transfer or elimination of heat from the exposed body surface, arising as the result of peripheral circulation, becomes negligibly small.

The fundamental need of the human body is, without question, to protect the innermost and therefore most vital organs, blood channels and tissues. Hence, as the surface of the body cools off, there is presumably a decrease in the amount of blood carried to the surface, so that ultimately the subcutaneous layer becomes virtually increased in depth and literally serves as a blanket to conserve the heat in the inner portions or, in other words, to lessen the rate of elimination of heat. Roughly speaking, such layers then act as insulating materials, possibly similar to cork, and as a result the effective temperature gradient is markedly affected. The foregoing statements offer some explanation as to why it is that, when at rest for some time and insufficiently clothed, we feel cold and clammy and why we often shiver. For without muscular exercise, which is associated with the increased production of heat, and without adequate means of decreasing the effective cooling surface, peripheral capillary stasis is induced with some constriction of the cutaneous blood vessels.<sup>4</sup> Shivering is a reflex form of muscular exercise and stimulates the peripheral circulation.

Before passing on, I emphasize that part of the routine in all the experimental work discussed in this paper, which consisted in keeping the subject without breakfast and at rest for at least one-half hour before an experimental test, and in making him sufficiently comfortable to insure few, if any, bodily movements, particularly of the extremity immersed in the calorimetric bath. Both of these precautions are quite necessary if useful results are to be obtained.

Furthermore, the curves of figures 3 to 8 inclusive and the data of table 3 show that the average inherent heat capacities (calories eliminated for each square inch each minute) are as follows: normal, 1.91; polycythemia, 2.17, and thrombo-angitis obliterans, 0.55. The average values for the rate of heat flow by reason of capillary activity are normal,  $43 \times 10^{-7}$ , polycythemia,  $114 \times 10^{-7}$ , and thrombo-angitis obliterans,  $22 \times 10^{-7}$  for each square inch each minute. It

<sup>4</sup> Some recent preliminary experiments by Sheard and Brown, on the effects of insertion of the hand for several minutes into an ice-cooled chamber, have shown microscopically that there is no such capillary constriction and apparently little, if any, dilatation, but that the rate of blood flow in the capillaries is considerably reduced, approximating a condition of stasis.

is not, of course, permissible to attach any undue significance to any average value obtained from the data from a few cases only. But these data substantiate, in a general way, the very conditions which should be expected a priori. For polycythemia is a condition of excess in the number of red corpuscles and in the blood volume. It would therefore follow that the number of active capillaries (and possibly size and rate of blood flow) would be in excess of that found in normal persons, hence giving a greater area of peripheral blood surface, and would therefore enhance the rate of flow of heat as compared with the rate for normal persons. However, the inherent heat capacity of the extremity, in an uncomplicated condition of polycythemia, would be increased but little, the whole mechanism of circulation being regulated to conduct and radiate heat from the surface at a greater rate, under a given temperature environment, than in the normal. This point is further substantiated by various microscopic and photomicroscopic studies of the capillaries in cases of polycythemia made by Brown, from the clinical and medical standpoint, and by my own physical data and experimentation. In such cases, following the reduction of blood volume the number of capillaries, visible and functioning in a given specified area, has been found to be very considerably reduced, although there are but slight, if any, changes in rates of blood flow in the capillaries, or in the calibers or lengths of the visible portions of capillaries.

#### SUMMARY

- 1 Calorimetric methods and data cannot be used to determine the quantity or rate of blood flow.
- 2 Only quantities ( $Q$ ) of heat and rates ( $K_1$ ) of transfer of heat can be examined in such calorimetric investigations, in which a temperature gradient ( $T_2 - T_1$ ) exists between the immersed extremity and the calorimetric bath.
- 3 The equation for heat conductivity stating that:

$$Q = E \cdot r - r_1 \frac{A}{D} \cdot t$$

4 The rate of increase ( $K_3$ ) of the temperature of the calorimeter and contents due to the peripheral or surface circulation is given by the expression

$$K_3 = \frac{1}{t_1 - t_2} \log_{10} \frac{T_2}{T_1}$$

5 Analyses of the experimental results made by the use of this equation in which  $\log_{10}\Delta T$  is plotted as the ordinate relative to the time  $t$  as abscissa, show that there are two distinct portions (a) that given by the transfer or elimination of heat by virtue of the temperature gradient existing between the foot and the calorimetric bath due to the inherent thermal capacity of the extremity plus the effects due to surface circulation, and (b) that given by the transfer or elimination of heat due solely to circulatory conditions at or near the surface

6 From a study of a normal person under various conditions of environmental temperature and under the conditions of experimentation stated, there is evidence that (a) the rate of transfer or elimination of the heat due to the surface or peripheral circulation *per se* is approximately very directly proportional to the temperature, in degrees Centigrade, of the surrounding environment, (b) the inherent thermal capacity of the superficial or surface layers of the extremity increases proportionately to the square of the temperature, in degrees Centigrade, of the surrounding atmosphere or environment, and (c) when the temperature of the surrounding environment falls to approximately 15°C, the rate of transfer of heat from the exposed surface of a resting body becomes negligibly small as is indicated by the value of the rate of heat elimination due to the existing conditions of surface circulation

7 A comparison of data on the inherent thermal capacities of extremities in selected normal subjects, three cases of polycythemia and four cases of thrombo-angitis obliterans (Buerger's disease), shows that there is but little difference in general between that in normal subjects and that in cases of polycythemia, but that there is a marked difference between the values in normal subjects and those obtained in cases of Buerger's disease

8 A study of the rates of elimination of heat at the surface of an

extremity, due to conditions of surface circulation, indicates that this rate of heat elimination was found to be from two to five times as great in the cases of polycythemia as in the normal subject under similar environmental temperature, and again, about half as great or less in cases of thrombo-angitis obliterans (Buerger's disease) as in normal subjects.

9 Tables and discussion of results are given in which the ratios of heat transferred in calories for each square inch of surface of extremity each minute, due to the inherent thermal capacity and the rate of elimination of heat by reason of the peripheral or surface circulation, are compared by various methods.

#### BIBLIOGRAPHY

- 1 Brown, G E Ann Clin Med, 1922, i, 69-77 Capillary Observations in Cardiovascular Renal Disease
- 2 Brown G E, and Giffin, H Z Am Jour Med Sc, 1923, clxvi, 489-502 Studies of Capillaries and Blood Volume in Polycythemia Vera
- 3 Cotton, T F, Slade J G, and Lewis, Thomas Heart, 1915-1917, vi, 227-247 Observations on Dermatographism with Special Reference to the Contractile Power of Capillaries
- 4 Krogh, August The Anatomy and Physiology of the Capillaries. New Haven Yale Univ Press, 1924, 276 pp
- 5 Mellor, J W Higher Mathematics New York, Longmans, Green and Company 1919, p 58
- 6 Sheard Charles Science, 1924, ix, 409-410 Laboratory Apparatus and Methods Instantaneous Photomicrography of the Skin Capillaries in the Living Human Body
- 7 Sheard, Charles, and Brown G E Jour Lab and Clin Med, 1924-1925, x, 925-929 A Method for Instantaneous Photomicrography of the Skin Capillaries.
- 8 Stewart G N Heart, 1911-1912 iii, 33-88 Studies on the Circulation in Man I The Measurement of the Blood Flow in the Hands. II The Effect of Reflex Vago-motor Excitation on the Blood Flow in the Hand
- 9 Stewart G N Harvey Lectures, 1912-13, 86-149 Studies on the Circulation in Man The Blood Flow in the Hands and Feet in Normal and Pathologic Cases
- 10 Taylor, N B Jour Lab and Clin Med, 1921-1922, vii, 439-463 The Blood Flow in Man as Estimated by the Calorimetric Method of Stewart.
- 11 Watson, W General Physics. New York Longmans, Green and Company, 1914, p 175



## CALORIMETRIC STUDIES OF THE EXTREMITIES

### II EXPERIMENTAL APPARATUS AND PROCEDURES<sup>1</sup>

By ROY KEGERREIS

(Received for publication July 1, 1926)

The calorimeter used in these experiments is a modification of that devised and used by Stewart (1). The ensemble of apparatus is shown in figures 1 and 2. The details of construction of the calorimeter are sketched in figure 3. Preliminary tests showed that the water equivalent of the calorimeter under the conditions of use was 170 gram calories. In the chamber *b* there is a false bottom of wire of coarse mesh on which the foot of the subject is allowed to rest. This arrangement permits of better stirring of the water around the foot and also minimizes the tendency on the part of the person under test to move the foot. The temperature of the water in the calorimeter was measured and read to 0.01°C by means of a certified Beckmann thermometer.

In order that the temperature may be kept as nearly uniform as possible throughout the calorimeter and therefore be correctly recorded by the thermometer, it is necessary that the water be stirred constantly and thoroughly during the period of the test. This stirring is accomplished by air currents in preference to any form of mechanical stirrer, not only because agitation by air is highly satisfactory, but also because of difficulties presented with any form of mechanical stirring.

The stream of air used for the agitation of the water in chamber *b* (fig. 3) must be at the same temperature as the water in this chamber and must enter it saturated with water vapor in order not to affect the temperature in the chamber. The apparatus used for this purpose was constructed and incorporated in the experimental ensemble as a

<sup>1</sup> This investigation was carried on while the writer was a member of the Section on Physics of The Mayo Clinic.

separate compartment *a* of the experimental calorimeter. It proved very reliable and easy to manipulate. Air drawn from the room is passed under pressure through a helical coil of copper tubing *X* immersed in water which is maintained as nearly as possible at the same

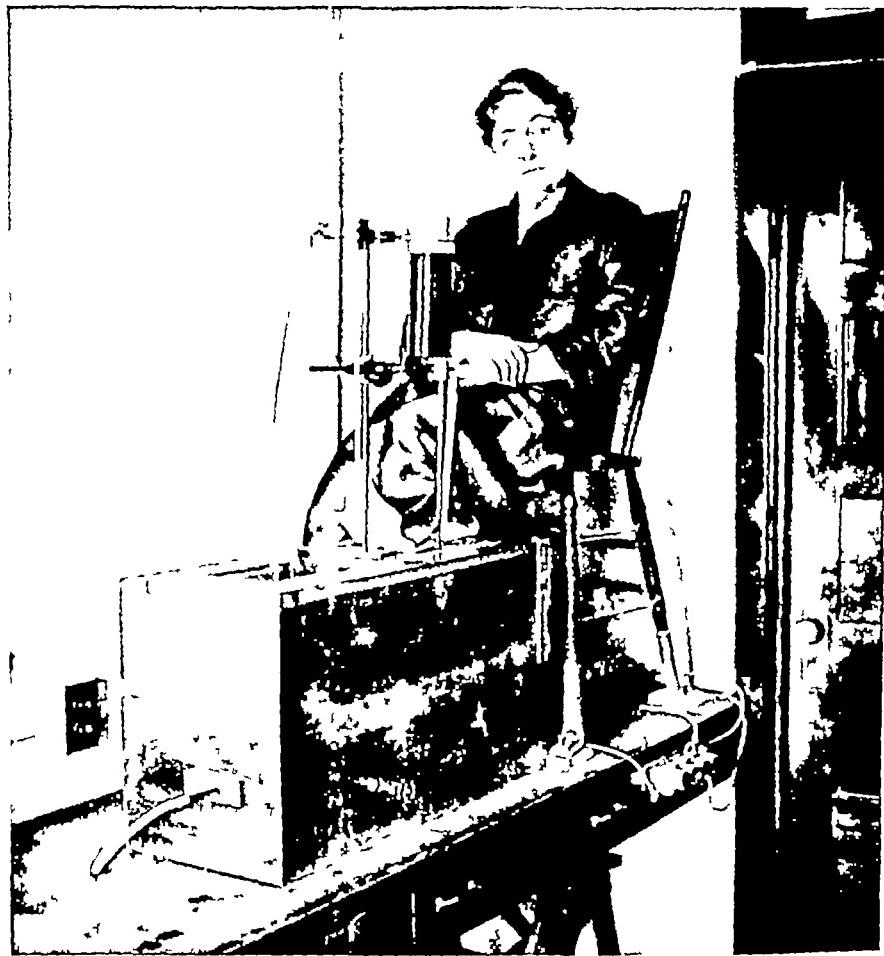


FIG 1 APPARATUS USED IN MAKING CALORIMETRIC STUDIES ON THE EXTREMITIES

temperature as that of the bath used for the immersion of the foot. The temperature of chamber *a* is regulated by electric bulbs controlled by a series rheostat. The copper coil is wound around the heating lamp as indicated by *R* (fig. 3). A current of air passing through it

keeps the "conditioning" bath, as it may be called, in chamber *a* thoroughly agitated. After passing through the coil *X* the air is allowed to bubble through the water in the container *Y*, this procedure saturates the air with water vapor and prevents loss of heat by evaporation in the foot bath. The chamber *Y* is immersed in the same auxiliary or "conditioning" bath as the coil *X*, hence the saturation of the air with water at the proper temperature is assured.



FIG. 2 ARRANGEMENT OF GALVANOMETER AND SCALE SO THAT EQUALITY OF TEMPERATURE OF WATER IN THE TWO COMPARTMENTS OF THE CALORIMETER MAY BE READILY OBTAINED WITH THERMOCOUPLES

A system of thermocouples inserted in the two chambers *a* and *b* connected to a sensitive galvanometer enables the operator to compare the temperatures of the water in the two compartments. The galvanometer, source of illumination and scale are shown in figure 2. The scale is made transparent so as to enable the operator to make the galvanometer reading from either side. When the temperatures are the same in both compartments there is no difference in electromotive force in the two sets of thermocouples, and hence no current flows through the galvanometer. Any difference in temperature of the

thermocouples, however, causes the galvanometer reading to fall on one side or the other of the previously determined zero point, indicating the amount of regulation of the heating device which is probably required. This regulation is secured by means of a multiple-stepped rheostat.

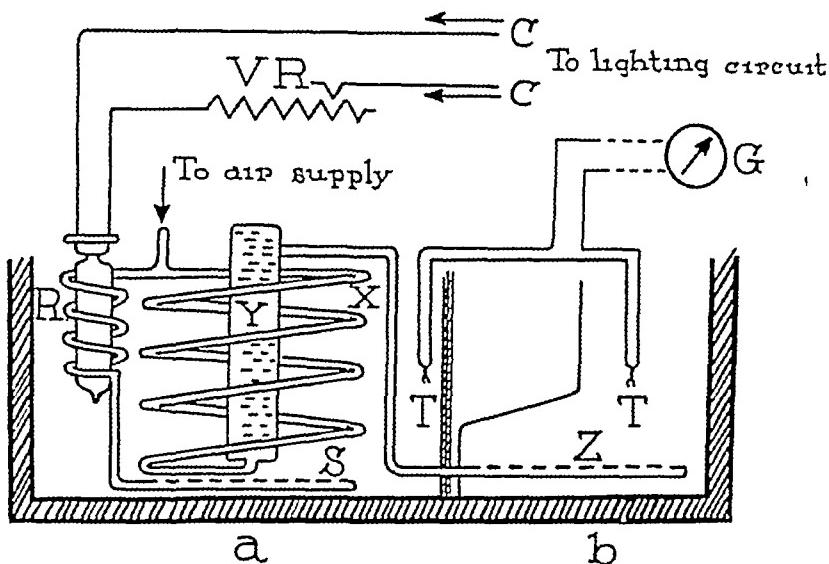


FIG. 3 DIAGRAMMATIC SKETCH OF CALORIMETER

*a* and *b* are the two compartments, *T*, *T*, thermocouples, *R* and *V R* variable resistance and heating circuit, *G*, galvanometer, *X*, *Y*, and *S*, apparatus for saturating air with water vapor, *Z*, device for stirring contents of chamber *b* with air from chamber *a*

#### EXPERIMENTAL AND CLINICAL PROCEDURES

Figure 4 gives a typical curve showing the relationship between the temperature of the calorimeter and the time of the test. The portion *CD* represents the conditions during the time prior to the setting up of a fairly uniform rate of increase of temperature. The outer portions of the foot cool off during this period, and this cooling supplies a large part of the initial rise in temperature in the calorimetric bath. The portion *DE* of the curve represents the condition of fairly uniform or steady rate of transfer of heat, here the inherent heat, or heat capacity of the foot, has a negligible effect, while the surface circulation is re-

sponsible for the heat which is eliminated from the extremity after it has been immersed in the bath for some minutes. It is highly essential that the conditions of transfer of heat which are represented by the approximately straight line of the graph shall prevail if any fairly reliable clinical index of surface circulatory conditions is to be secured.

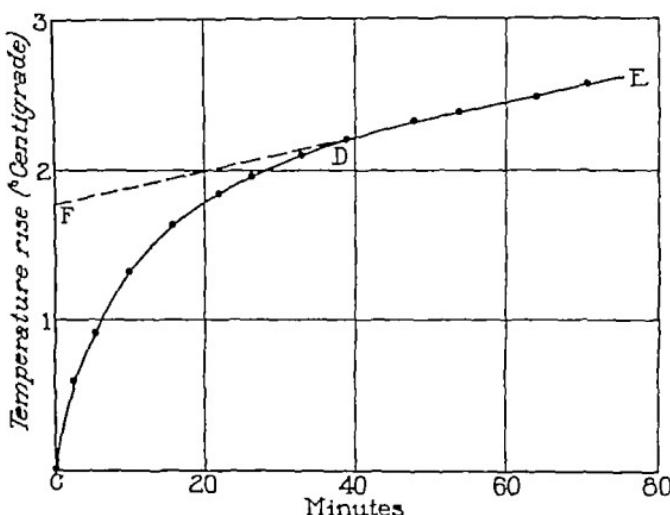


FIG. 4 A TYPICAL CURVE SHOWING THE RELATIONSHIP BETWEEN THE RISE OF TEMPERATURE OF THE CALORIMETER AND CONTENTS AND THE TIME OF THE TEST

The portion of the curve *ED* represents the condition of a fairly uniform rate of transfer of heat.

A close approximation to the heat given out by the foot, other than by virtue of the superficial or peripheral circulation, may be obtained by extending the line *DE* back to the point *F* which intercepts the axis of temperature at the time at which the experimental test was started. The ordinate *CF* of this intercept indicates with fair accuracy the rise in temperature of the calorimeter due to the cooling of the tissues at or near the surface of the extremity. The approximation involves the supposition that the conditions of surface circulation have remained constant throughout the interval. All tests were continued until

the graph, in each instance, had approximated a straight line, for at least twenty minutes

The temperature of the calorimeter and its contents at the start of a test was always made the same as that of the room. This temperature was held, so far as possible, at about 22°C. It is, of course, necessary to correct for the cooling of the calorimeter as it warms up above the surrounding atmosphere. The cooling curve from which corrections have to be made very closely approximates a straight line for small differences of temperature. Twenty minutes was arbitrarily selected as the time interval to be considered in all tests. It is then necessary to know how much heat the calorimeter has lost during the same twenty minutes in order to make the proper correction, which may under certain circumstances be a considerable proportion of the heat transferred.

A gauge, shown in figure 5, was constructed to facilitate the rather laborious transfer of graphical data with the necessary correction into a numerical index. The gauge greatly facilitates the work and involves no added approximations. It was especially designed for the work in hand and consists of two parts. The upper part is made of transparent celluloid and slides up and down over the lower part which is heavy, lies flat and is covered with coordinate paper on which are plotted cooling curves for the metal boot of the calorimeter.

In use, the left edge of the lower part of the gauge is laid on the graph sheet vertically and opposite the time which marks the end of the twenty-minute interval in such a way that the temperature scales of the graph and gauge correspond. The transparent part is then moved up or down until the fiducial mark at the lower left-hand corner lies on the graph.

The amount that the graph is raised during a period of twenty minutes gives a direct measure of the heat transfer, but without any consideration of correction for the loss of heat to the surroundings. A cooling curve for the average differences of temperature during twenty minutes gives the necessary correction. The lower stationary part of the gauge has on it these correction curves for cooling for periods over twenty minutes.

The average temperature should be the basis for any cooling correction. A new setting is required to accomplish this, the lower part

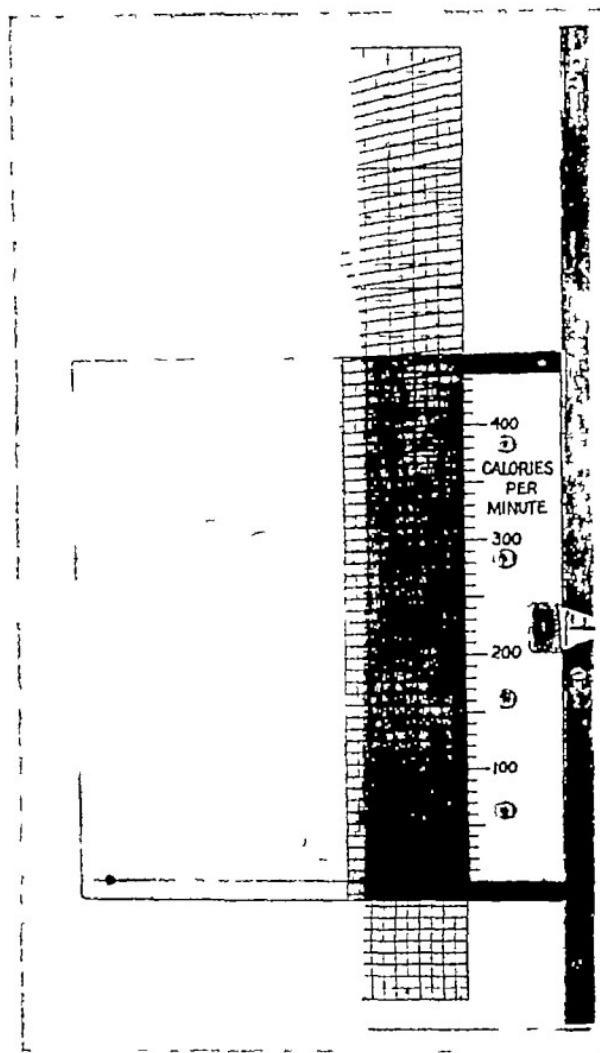


FIG 5 A GAUGE TO FACILITATE THE TRANSFER OF GRAPHICAL DATA INTO CALORIES PER SQUARE INCH PER MINUTE

of the gauge is raised half the amount which the temperature has increased in the twenty minutes. The upper celluloid is then readjusted so that the fiducial mark rests in the time-temperature graph, and the correction curve ther makes the necessary adjustment based on the average difference in temperature. An ordinary approximation to the average temperature will serve when the gauge is being set, since nearby correction curves are quite similar.

In description, the process is technical and appears laborious, but in practice it is simple and rapid, only a moment being required to secure

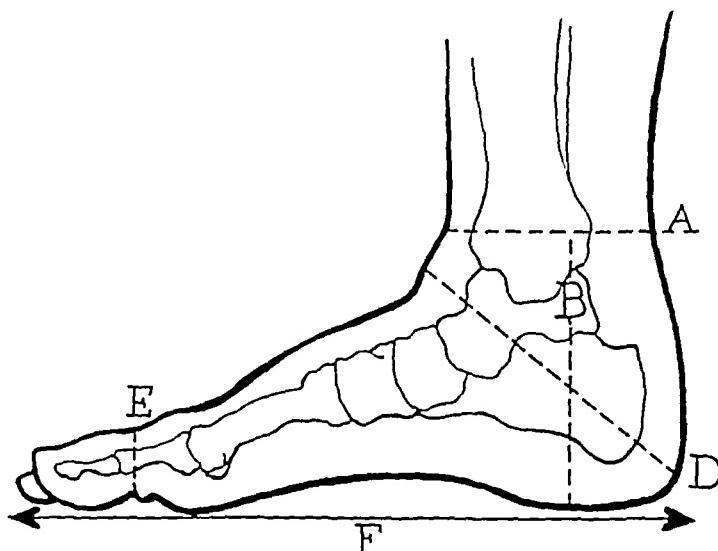


FIG 6 DIAGRAMMATIC SKETCH OF THE MEASUREMENTS OF THE FOOT NECESSARY TO PERMIT THE CALCULATION OF THE AREA OF THE IMMERSSED EXTREMITY BY A MATHEMATICAL FORMULA (E, D AND A ARE CIRCUMFERENCES)

the reading with due correction for the loss of heat to the surroundings. The correction curve is followed to the right to the scale which gives the calories transferred during the twenty-minute period. The scale is so calibrated that the calories each minute are given and the necessity of dividing by twenty is also avoided. This, then, is the total amount of heat transferred, and when divided by the area of the foot gives the amount of heat transferred each minute for each square inch of skin on the foot. The heat transferred obviously depends on the area exposed.

There is such a variation in the size of feet, and consequently the depth of immersion, that it was decided to reduce all readings to unit area of skin exposed. Figure 6 shows the measurements which are taken on each foot. The area is computed by means of a formula



FIG. 7 PARAFFINED STOCKING USED TO MEASURE THE AREA OF THE FOOT

which was evolved from twenty three actual determinations. The formula is

$$\begin{aligned}\text{Foot area} &= 0.31F(E + 3/4D) + 0.775B(A + 3/4D) - 0.124D^2 \\ (\text{in square inches}) \quad &= 1.25(\text{Vol in cc of extremity}) + 2000(0.00147 - B)A\end{aligned}$$

The actual areas were determined by drawing a tight fitting lisle stocking over the foot after it had been covered with thin tissue paper. Paraffine, which was just above its melting point, was then slowly applied with a brush and allowed to cool. Figure 7 shows such a stock-

ing after its removal, and figure 8 shows how it was cut in order to flatten or lay it out on a large sheet of photographic paper. The area of the paraffine stocking was secured by the method of weighing after a photographic print had been made.



FIG. 8 THE PARAFFINED STOCKING LAID OUT FOR MEASUREMENT

#### DISCUSSION

Calculations on the volume of blood flow have not been made, since the conductivity of the skin, the number of functioning capillaries, and areas of exposed blood are also important and somewhat indeterminable factors which vary in different individuals. The number of calories transferred for each unit area of skin each minute is taken

as the final index of heat transfer. These, I believe to be the only data which can be obtained and correctly interpreted, for one can obtain experimental data on rates of elimination of heat which are of value from both physiologic and pathologic viewpoints, and which do not involve any assumptions relative to the possibility that these rates of transfer of heat are direct measurements on the rates of flow of blood. The time required for a constant rate of transfer of heat to be reached varies much in individual cases, and this variation indicates quite clearly that one or more of the factors entering into these experimental studies are not the same in different individuals.

To the first order of approximation, it may be said that the change of temperature in the calorimeter represented by the ordinate  $CF$  (on the time temperature graph of figure 4, for example) is due to the inherent heat in the tissues in the outer layers of the extremity immersed in the bath. The heat thus eliminated, when calculated and divided by the area of the extremity and the specific heat of the tissues, gives an approximate measure of the half-depth to which the superficial layers have been cooled. The determination of the half depth to which the outer layers of an extremity have been cooled to the temperature of the calorimetric bath may be an index of the conditions of circulation of the blood in an extremity and may prove of value in future studies on these problems.

#### BIBLIOGRAPHY

- 1 Stewart, G. N. Heart, 1911-1912, III, 33. Studies on the Circulation in Man  
Also Harvey Lectures, 1912-1913, 33.



## CALORIMETRIC STUDIES OF THE EXTREMITIES

### III CLINICAL DATA ON NORMAL AND PATHOLOGIC SUBJECTS WITH LOCALIZED VASCULAR DISEASE

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Minnesota)

(Received for publication July 1, 1926)

#### INTRODUCTION

The clinical investigation of the different forms of vascular disturbances involving the extremities has been greatly hampered by the lack of exact methods for measuring the volume flow of the blood. The classic descriptions of Raynaud, Weir Mitchell, Buerger, and others, on the different forms of the localized vascular diseases leave little to be added from the purely clinical side. There is no doubt that the signs and symptoms of these disturbances are of the utmost importance and furnish information on which the final word in diagnosis, prognosis and treatment must largely rest. Data on the volume flow of blood in a given mass of tissue would be a most valuable aid in determining the degree of vascular obliteration, the amount of collateral circulation, and the vasomotor impairment, and in evaluating the different forms of treatment. The plethysmographic studies of Hewlett and Van Zwaluwenburg (5) and the calorimetric investigations of Stewart and Taylor (6, 7) have demonstrated the applicability of instrumental methods in the investigation of vascular disturbances.

The present work represents an extension of Stewart's calorimetric method in the investigation of vascular diseases. Further information has been sought on the following questions: (a) Can the values for the elimination of heat from the limb be accurately transposed into terms of volume flow of blood? (b) What is the average range of elimination of heat or volume flow of blood of the extremity in the normal subject and in the subject with obstructive vascular lesions?

of the extremities? (c) What are the effects of environmental temperature on the rate of elimination of heat in the extremity in the normal subject and the subject with vascular disease affecting the extremities?

#### METHOD OF INVESTIGATION

The calorimeter<sup>1</sup> is a container holding a known volume of water of a lower temperature than the skin, into which the hand or foot is placed. It is so constructed that the loss of heat is slight, and the corrective value is known. Certain technical improvements have been made by Kegerreis. For example, an adequate mixing device for the proper distribution of heat and water has been incorporated in the calorimeter. The method follows closely that outlined by Stewart. The temperature of the calorimeter and its contents is allowed to come into equilibrium with that of the room, which is maintained as closely as possible between 22° and 24°C. The foot is immersed and the temperature of the water noted at intervals of five minutes. The readings are tabulated and plotted on graph paper against the time readings of the experiment. They are continued long enough so that four consecutive readings, when plotted and joined, approximate a straight line. This would indicate a linear or constant relationship between the rise of temperature of the water and the time of the immersion period. A curve consisting of two portions is thus constructed. The first or rapidly rising portion represents largely the loss of *a*, the metabolic and inherent tissue heat, and *b*, the heat given off from the surface blood.<sup>2</sup> Vasomotor equilibrium is probably established during this initial immersion period. The second portion of the curve is approximately a straight line representing the period of steady transfer of heat, and is taken to represent that due mainly to the steady loss of heat from the surface blood (fig. 4, Study II). This portion of the curve is considered to furnish the significant data and has been utilized as the basis of these studies. The rise in temperature of a known volume of immersion

<sup>1</sup> The complete technical description of the calorimeter is given by Kegerreis in Study II of this series.

<sup>2</sup> The inherent heat or thermal capacity of the foot represents the heat remaining in the tissues after the arterial flow is checked.

water (4,000 cc.) for a period of twenty minutes is then converted into small calories and the rate of heat elimination is determined in calories for each minute for each square inch of surface area<sup>2</sup>.

*Application of calorimetric data.* Sheard in his studies has shown the impossibility of interpreting the loss of heat from the extremity in terms of volume flow of blood. Accurate data on the volume flow of blood, although most desirable, is not essential. The amount of heat eliminated for a unit of surface area is probably of equal clinical value, since there is a close relationship between volume flow of blood and loss of heat. Too many unknown factors exist to attempt to define this relationship. The problem then, from the clinical standpoint, was to determine (a) the average range of heat elimination in small calories in the feet in a group of normal subjects, (b) the effects of environmental temperature on the rate of heat elimination, and (c) the range of heat elimination in a group of patients with proved obstructive arterial disease, that is, thrombo-angitis obliterans. It was felt that comparison of the data of the normal and pathologic subjects would determine the diagnostic value of this method, especially when controlled by studies on the effect of different environmental temperatures on the rate of heat elimination.

*Routine of determinations.* The ambulant subjects were allowed to rest in the sitting posture with feet bared, in the calorimetric room for a period of thirty minutes. This preliminary step was found to be unnecessary for the patients in the hospital. The usual diet was allowed. The actual room temperature during the majority of determinations varied from 22° to 26°C. When repeated determinations were carried out on the same foot, an intermission period of at least one hour was allowed. After shorter intermissions the rate of loss of heat was lower, as would be anticipated from the cooling of the skin by the immersion and subsequent evaporation.

*Precautions necessary in determinations.* Muscular movement in the immersed foot increases the rate of liberation and elimination of heat so the patient is instructed to keep the foot quiet and relaxed. A comfortable sitting position for the patient is necessary as

<sup>2</sup>The area of the foot is determined by a method devised by Kegerreis and described in Study II. The area in square inches can be converted to square centimeters by multiplying by 6.45.

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*Application of calorimetric data* Sheard in his studies has shown the impossibility of interpreting the loss of heat from the extremity in terms of volume flow of blood. Accurate data on the volume flow of blood, although most desirable, is not essential. The amount of heat eliminated for a unit of surface area is probably of equal clinical value, since there is a close relationship between volume flow of blood and loss of heat. Too many unknown factors exist to attempt to define this relationship. The problem then, from the clinical standpoint, was to determine (a) the average range of heat elimination in small calories in the feet in a group of normal subjects, (b) the effects of environmental temperature on the rate of heat elimination, and (c) the range of heat elimination in a group of patients with proved obstructive arterial disease, that is, thrombo angiitis obliterans. It was felt that comparison of the data of the normal and pathologic subjects would determine the diagnostic value of this method, especially when controlled by studies on the effect of different environmental temperatures on the rate of heat elimination.

*Routine of determinations* The ambulant subjects were allowed to rest in the sitting posture with feet bared, in the calorimetric room for a period of thirty minutes. This preliminary step was found to be unnecessary for the patients in the hospital. The usual diet was allowed. The actual room temperature during the majority of determinations varied from 22° to 26°C. When repeated determinations were carried out on the same foot, an intermission period of at least one hour was allowed. After shorter intermissions the rate of loss of heat was lower, as would be anticipated from the cooling of the skin by the immersion and subsequent evaporation.

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<sup>3</sup>The area of the foot is determined by a method devised by Kegerreis and described in Study II. The area in square inches can be converted to square centimeters by multiplying by 6.45.

the experiment frequently lasts an hour or longer. Changes in room temperature due to the opening and closing of doors should be obviated as much as possible. Environmental temperature proved to be the most disturbing factor in making determinations. During the period of excessively hot weather comparative determinations were impossible. High rates of elimination of heat were obtained in the normal controls. It was found that when the outdoor temperature was high and the room temperature within working range, normal persons maintained increased heat values for variable periods of time after coming indoors. All data recorded during the hot weather have been discarded except those necessary to determine temperature effects. The barometric pressure seems to exert no significant influence on heat elimination.

#### MATERIAL STUDIED

*Normal group (table 1)* Comparative calorimetric studies of the feet were carried out under controlled conditions in twenty-two normal persons whose ages varied from fifteen to fifty-four years. The incidence of males and females was about equal. Repeated daily determinations in a normal boy, aged sixteen, were carried out for four days in August (table 2). The outdoor temperature during this period ranged from 22.2° to 28.6°C with approximately the same fluctuations indoors. A similar series of determinations was made on a normal girl during the latter portion of the same month (table 3). The outdoor temperature ranged from 18.2° to 27.4°C, with closely approximating values for the room temperature. The pulse rate, blood pressure and mouth temperature were noted.

*Pathologic group (table 4)* Sixteen cases of thrombo-angitis obliterans (Buerger's disease), four cases of Raynaud's disease affecting the feet, and seven cases of spastic paraplegia were studied. The clinical diagnosis of Buerger's disease rested largely on the age, sex and history of the patient, and on the lack of calcification in the arteries as determined by the roentgen ray. Each patient showed definite evidence of a chronic obliterative lesion of the main arteries of the feet, as no pulsation was felt in the vessels. This observation was confirmed in oscillometric readings with the apparatus of Pachon. All these patients showed varying degrees of trophic disturbances,

and about half had frank gangrene. The feet were cold, and all showed the characteristic redness of the skin accentuated by allowing the feet to assume the pendent position. In six cases amputation was performed subsequently and the characteristic pathologic lesions of this disease were found.

#### RESULTS

*Normal subjects.* Table 1 shows the range of loss of heat from the feet in ambulant and hospital subjects as measured in calories for each minute for each square inch of surface area. These data demonstrate that with the room temperature varying from 21.2° to 29.1°C the heat eliminated during the initial determination varies widely (from 0.35 to 3.89 calories). With the room temperature between 22° and 26°C the range of loss of heat is more restricted, varying from 0.46 to 1.15 calories for each minute for each square inch of surface area. The outdoor temperature varied from 5.2° to 32.1°C. The lowest values for loss of heat were obtained during the period of low outdoor temperatures.

Repeated determinations were made in one subject (case 10) on the right foot with the room temperature varying from 21.8° to 23.9°C and the outdoor temperature varying from 5.2° to 19°C. Marked variations in the rate of loss of heat were found, the lowest rate was 0.13 and the highest 0.54 calories. The lower rates were obtained with low outdoor temperatures.

In figure 1 the heat in calories lost each minute for each square inch of surface area in a larger series of cases is plotted against room temperature. It will be observed that in the majority of determinations within the temperature range of 21.5° to 26.0°C, the values for loss of heat vary from 0.4 to 1.5 calories. It is noted that the relationship between the loss of heat and room temperature is roughly a linear one with the room temperature between 21.5° and 27.0°C. Above this temperature there is less loss of heat with a flattening out in the theoretic curve. In these experiments the variation in the rates of elimination by normal subjects is 350 per cent, under a restricted environmental temperature (21° to 26°C). Subjects examined during the cooler months of the year and under hospital restrictions have shown a narrower range of heat elimination.

Determinations on the same subject with comparable room temperatures give quite closely approximating values. Repeated determinations on the rate of heat elimination on the feet of two normal subjects, a girl and a boy, aged seventeen and sixteen, respectively, were made over a period of a month when there were weather varia-

TABLE 1  
*Calorimetric determinations in right foot of normal subjects at rest*

Case	Sex	Age	Blood pressure		Pulse rate	Temperature			Total calories lost in twenty minutes	Surface area of foot	Calories lost each minute for each square inch
			Systolic	Diastolic		Mouth	Outdoor	Room			
1	Γ	26	115	70	74	98 4	24 0	24 0	1,160	94	0 61
2	M	34	120	70	72	98 6	18 8	22 4	2,240	112	1 00
3	Γ	20	92	58	80	98 2	22 1	24 7	1,800	87	1 03
4	M	15	124	90	82	98 8	14 1	22 0	2,340	101	1 15
5	M	50	114	70	78	99 5	37 2	22 8	1,800	79	1 14
6	Γ	40	114	70	82	98 0	36 8	22 2	1,700	87	0 97
7	Γ	27	106	62	70	98 8	7 8	21 2	1,100	77	0 71
8	M	28	104	58	68	97 8	7 8	22 2	940	111	0 42
9	M	51	105	92	72	98 2	31 0	30 4	1,600	100	0 80
			130	76	88	99 0	19 0	22 6	1,080	101	0 53
10	M	27	120	80	80	98 2	6 8	23 9	1,100	101	0 54
					80	98 0	5 2	21 8	280	101	0 13
					88	98 4	6 0	23 6	460	101	0 23
11	M	28	110	65	88	99 4	30 2	28 7	1,560	113	0 69
12	Γ	42	110	70	80	98 2	24 3	23 4	1,040	75	0 70
13	M	52	145	98	70	97 8	29 7	29 1	7,400	90	3 89
14	Γ	45	120	82	72	98 2	26 0	26 0	1,060	80	0 66
15	M	54	150	90	72	98 0	8 9	22 6	960	103	0 46
16	Γ	53	140	100	74	98 2	6 8	21 2	580	82	0 35
17	M	28	110	65	88	99 4	30 2	28 7	1,560	113	0 69
18	M	28	122	68	86	98 4	32 1	27 2	860	113	0 38
19	M	28	132	66	120	98 6	18 8	22 5	980	113	0 43
20	M	22	140	80	60	98 6	14 5	22 0	700	102	0 46

tions of  $15^{\circ}\text{C}$ . The female subject, although classed as normal, was representative of the group of persons of the mild asthenic type with cool moist hands and feet who actually have abnormal vasomotor control. The difference between the temperature of the mouth and surface of the foot varied from  $8^{\circ}$  to  $12^{\circ}\text{C}$ . The blood pressure was

slightly lowered. The calorimetric determinations from August 8 to 28 were fairly low, varying from 0.18 to 0.77 calories (table 2). These values existed with a fairly constant room temperature ranging from 20.3° to 25.3°C. Determinations in both feet during the same day showed remarkably close values. During the afternoon of August 28 the room temperature increased 3.4°C and the rate of heat elimination increased from 0.65 to 1.5 calories. The lower values of loss of heat were obtained on the cooler days and higher values during the periods of warmer weather. Repeated determinations on August 12 with temperature changes of 2.3°C, or of an increase of

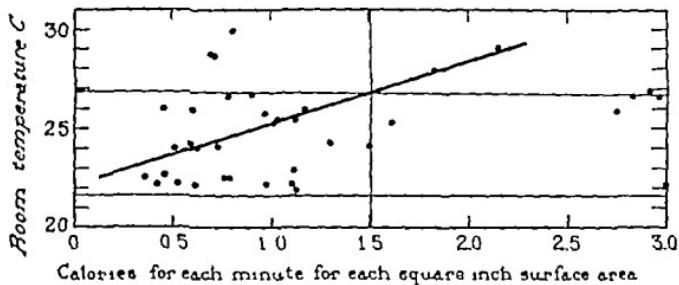


FIG. 1 RELATIONSHIP BETWEEN ROOM TEMPERATURE AND RATE OF HEAT ELIMINATION IN NORMAL SUBJECTS

Between 21.6° and 26°C it is approximately linear. Each point represents the rate of heat elimination in a normal subject, shown on the abscissa, plotted against environmental temperature, shown on the ordinate.

10 per cent in the outdoor and room temperatures, showed an increase of 70 per cent in the loss of heat. On August 28 there was an increase of 2.7°C, or 10 per cent in the outdoor temperature, and the rate of loss of heat increased 130 per cent. The following day, without change in the outdoor or room temperature, the rate of heat elimination increased about 100 per cent.

In the second series of determinations, which was carried out on a normal boy, aged sixteen, the feet were warm, from 4° to 6°C less than the mouth temperature (table 3). The initial calorimetric determinations showed a range of 0.52 to 2.97 calories for each square inch of surface area for each minute with the environmental

TABLE 2  
Repeated calorimetric determinations in normal girl, aged sixteen

Date	Blood pressure Systolic	Diastolic	Pulse rate	Temperature			Foot	Total calories lost in twenty minutes	Surface area of foot	Calories lost each minute for each square inch	Remarks
				°F Mouth	°C Outdoor	°C Room			square inches		
<i>1924</i>											
August 11	114	98	78	97.4	18.2	21.5	Right	540	90	0.30	At rest
	110	80	76	97.4	19.8	21.7	Left	440	89	0.24	At rest
			80	97.6	22.6	22.6	Right	560	90	0.31	At rest
August 12	108	80	82	96.6	20.2	21.8	Right	660	90	0.36	At rest
	106	60	90	97.6	21.6	22.3	Left	640	89	0.35	At rest
			88	98.1	22.5	23.1	Right	940	90	0.52	At rest
August 13	104	60	72	97.4	16.6	21.0	Right	340	90	0.18	Change in weather
August 15	110	64	84	98.0	19.4	20.3	Right	880	90	0.48	Twenty minutes after walking for fifteen minutes
August 27	110	74	74	98.0	23.2	23.5	Right	1,400	90	0.77	Change in weather
August 28	100	66	72	98.0	24.6	23.7	Right	1,180	90	0.65	At rest
	98	50	76	98.0	25.0	25.3	Left	1,200	89	0.67	At rest
			76	98.2	27.3	27.1	Left	2,800	89	1.3	At rest
August 29	95	60	74	98.0	27.4	24.9	Left	5,140	89	2.85	At rest

TABLE 3  
*Repeated calorimetric determinations in a normal boy, aged sixteen*

Date	Blood pressure		Pulse rate	Temperature			Foot	Total calories lost each minute for each square inch	Surface area of foot	Calories lost each minute for each square inch	Remarks
	Systolic	Diastolic		Mouth	Outdoor	Rooms					
1944											
August 4	136	92	96	99.6	28.6	28.2	Right	5,300	116	2.71	Hot sultry day
August 5											
	70	98.3	25.0	24.3	Right	2,940	116	1.31	Cool period of morning		
	72	98.4	25.4	25.4	Left	2,240	115	0.97	Immediately after test of right foot		
	70	98.4	26.4	26.1	Right	6,200	116	2.67	After rest of thirty minutes		
					Right	2,060	116	0.90	Fifteen minutes after previous test		
August 6											
	68	98.0	22.9	25.1	Right	2,400	116	1.03	After rest of thirty minutes		
	72	97.0	22.8	25.0	Left	1,720	115	0.75	Immediately after previous test		
	56	97.0	22.8	22.5	Right	1,420	116	0.61	After walking 504 steps		
	60	97.0	22.2	22.7	Right	900	116	0.36	After rest of thirty minutes and decrease in room temperature		
August 7											
	110	80	72	98.0	23.8	24.1	Right	1,760	116	0.76	After rest of thirty minutes
		84		23.4	24.2	Left	1,380	115	0.60	Immediately after previous test	
		60		26.1	25.5	Right	1,400	116	0.60	Twenty five minutes' rest after walking	
August 8											
	72	97.0	23.6	24.0	Right	1,220	116	0.52	After rest of twenty five minutes		
	70	98.5	26.2	25.5	Left	1,060	115	0.46	Immediately after test of right foot		
	70	98.5	25.8	25.8	Right	6,760	116	2.91	After rest of twenty five minutes		
	68				Right	1,840	116	0.79	Following preceding test		

temperature varying from  $22.5^{\circ}$  to  $28.2^{\circ}\text{C}$ . Four determinations were carried out August 5. The outdoor temperature and room temperature ranged from  $24.3^{\circ}$  to  $26.6^{\circ}\text{C}$ . The previous day had been extremely sultry with an outdoor temperature of  $28.6^{\circ}\text{C}$ , and the heat eliminated from the right foot was 2.7 calories for each square inch for each minute. The first determination showed a rate of 1.31 calories in the right foot and 0.97 in the left. During the afternoon the outdoor and room temperatures were rising, and the right foot gave off 2.6 calories at the first determination and 0.9 calories at the second. Fifteen minutes intervened between the two determinations. The heat lost by this foot showed a variation of about 100 per cent between the morning and afternoon determinations, with a variation of environmental temperature of  $2.1^{\circ}$  or about 10 per cent. The first determinations were made during the early morning. Calorimetric studies made on the same foot after previous immersion for from thirty to sixty minutes showed a sharp reduction in the loss of heat as shown August 5. The next day the outdoor temperature had decreased  $3.7^{\circ}\text{C}$  in twenty-four hours but the room temperature was still high, although it decreased gradually during the day, with a corresponding decrease in the rate of heat elimination. August 6 the outdoor and room temperatures increased about 10 per cent and the rate of elimination of heat in the right foot increased 600 per cent.

It will also be observed that in both of the normal subjects lower values were obtained for the left than for the right foot. The determinations were always carried out on the right foot first. This is no doubt a vasomotor effect of the cooling of the opposite extremity, the contralateral vasomotor response as noted by Stewart.

*Pathologic subjects.* Table 4 shows the data on the patients having thrombo-angiitis obliterans with obstructed arteries of the feet. The rate of heat elimination varied from 0.27 to 1.0 calorie with environmental temperatures ranging from  $21.8^{\circ}$  to  $26^{\circ}\text{C}$ . Repeated determinations gave similar results (table 5). It will be observed that with wide variation in the environmental temperature, the rate of heat elimination was more fixed and constant than in the normal subjects (case 4, table 5). A variation of  $5.2^{\circ}\text{C}$  occurred in the room temperature and the rate of loss of heat was unchanged in the right

foot. No clear-cut difference in the heat values was noted in the patients in the early and late stages of the disease.

*Comparative determinations for evaluation of treatment.* A large series of determinations have been carried out in cases of thrombo-angitis obliterans to study the effects of various forms of treatment. The rates of heat elimination have been followed before and after

TABLE 4  
*Calorimetric determinations of loss of heat in the feet of subjects with thrombo-angitis obliterans*

Case	Sex	Age	Blood pressure		Pulse rate	Temperature			Foot	Total calories lost in twenty minutes	Surface area of foot	Calories lost each minute for each square inch
			Systolic	Diastolic		Mouth	Out door	Room				
		years				F	C	C			square inches	
1	M	28	122	84	66	98.2	27.5	26.0	Right	1,520	112	0.67
2	M	45	108	76	80	97.4	25.2	25.5	Right	1,760	88	1.00
3	M	41	100	66	70	98.4	24.7	25.5	Left	1,540	97	0.79
4	M	36	110	76	80	98.4	13.7	24.0	Right	1,580	102	0.77
5	M	26	130	66	86	99.4*	9.2	24.0	Left	7,400	118	1.13
6	M.	45	110	85	80	98.5	-1.6	22.2	Left	1,520	110	0.69
									Left			0.64
7	M.	42	120	78	80	97.6	0	22.0	Left	640	93	0.39
									Left			0.30
8	M	37	122	86	86	98.0	-9	21.9	Left	1,300	113	0.57
9	M	42	94	60	60	98.0	-4	21.8	Left	1,100	99	0.55
10	M	37	101	68	78	97.8	-4.6	22.6	Left	640	104	0.30
									Right	540	100	0.27
11	M	45	128	82	80	98.6	-3	23.0	Left	1,480	112	0.66
									Left			0.54
12	M	49	126	86	82	96.0	6	22.3	Left	1,080	117	0.46
13	M	39	102	64	68	97.0	-4	22.0	Right	880	102	0.43
14	M	33	90	54	84	97.8	12.3	22.0	Left	1,000	101	0.50
15	M	50			70	97.0	11.1	22.1	Right	2,400	113	1.06
16	M	44	120	60	76	97.5	23.2	23.2	Right	1,480	108	0.68

\* Slight fever

intravenous injection of sodium citrate, hypertonic salt solution, and radium chloride, and after the oral ingestion of large quantities of Ringer's solution. No changes in the rate of loss of heat could be shown, although varying degrees of relief from pain were noted. Table 6 shows the rates of heat elimination of the feet in three cases of spastic paraplegia in which lumbar ganglionectomy was per-

TABLE 5  
*Comparative determinations of loss of heat of the feet in cases of thrombo-angiitis obliterans\**

Case	Date	Sex	Age	Blood pressure		Pulse rate	Temperature			Foot	Surface area of foot square inches	Calories lost each minute for each square inch
				Systolic	Diastolic		Mouth	Outdoor	Room			
1	1924						°F	°C	°C			
	June 30	M	49	116	80	85	97.0	16.9	22.0	Right	1,680	98 0.85
	July 1		142	88	89	97.8	17.8	21.8	Left	1,880	105 0.85	
	July 7		118	80	89	98.0	18.5	21.8	Left	1,420	105 0.67	
	July 9		116	78	72	98.2	25.0	24.4	Left	1,000	105 0.47	
2	July 25	M	30	112	80	90	98.0	25.4	24.8	Left	1,180	108 0.52
	July 29		104	64	87	99.0	24.8	24.6	Left	1,100	108 0.51	
	August 11		114	88	80	98.4	23.4	22.8	Left	920	108 0.42	
3	November 28	M	37	122	86	86	98.0	-9.5	21.9	Left	1,300	113 0.57
	1926											
	April 27		114	68	72	97.0	3.0	22.4	Left	1,400	98 0.76	
4	1925											
	June 1	M	43	128	78	78	98.6	27.6	27.2	Right	2,000	105 0.95
	June 5		120	68	98	99.0	28.7	27.8	Left†	5,660	97 2.91	
	June 8		118	68	76	97.4	16.8	22.3	Left	2,300	97 1.18	
	January 27					78	98.0	-14.0	22.0	Right	2,120	105 1.00

\* Note the relative constancy of the heat elimination  
 † No vascular occlusion in left foot

formed by Adson for its effect on muscle tone (4). Large increases in the rate uniformly followed operation. In consequence of these observations this operation was tried by Adson (1) in cases of Raynaud's disease to relieve the vasospastic disturbance, and large increases in the loss of heat were noted. In cases of thrombo angitis obliterans,

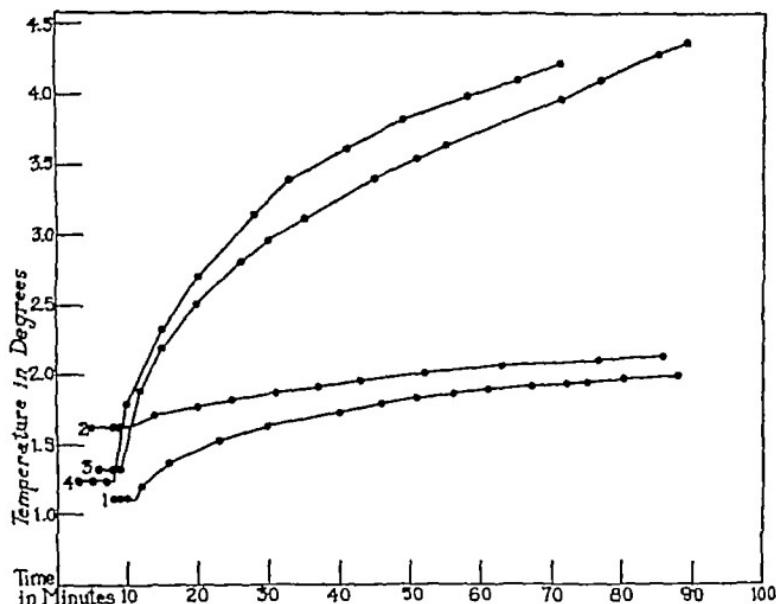


FIG 2. CURVES OF HEAT ELIMINATION IN NORMAL AND PATHOLOGIC SUBJECTS

- Curve 1 Raynaud's Disease
- Curve 2 Thrombo-angitis obliterans
- Curve 3 Normal Subject
- Curve 4 Erythromelalgia

Note the close parallelism in loss of heat between Raynaud's disease and thrombo-angitis obliterans.

increased vasodilatation and increased loss of heat were noted, although not to the degree observed in the non-obliterative types of vascular disturbance. The injection of intravenous protein (typhoid bacilli) produced large but temporary increases in the rate of heat elimination in certain cases of Buerger's disease (table 6).

Comparative determinants of loss of heat in the feet following surgical and medical treatment

Case	Sex	Age	Diagnosis	Date	Temperature				Foot				Remarks			
					Systolic	Diastolic	Pulse rate	Respirations	Right	Left	Right	Left	Right	Left	After	Before
1	F	28	Spastic paraplegia	1925	130	80	100	97 9 0	23 3	Right	560	69	0 40	Before operation		
				February 24	128	80	85	97 7 -9	319 0	Right	400	69	0 29	Before operation		
				February 25	84	97	9	4 22 2	2	Right	1,480	69	1 07	After lumbar neurectomy		
				March 19	114	86	72	97 7 21	722 4	Right	1,760	69	1 13	After lumbar neurectomy		
2	M	43	Spastic paraplegia	1925	128	80	68	97 0	21 7 24 0	Left	1,400	95	0 73	Before operation		
				March 9	70	70	97 9	21 7 24 0	Left	1,360	95	0 71				
				April 6	64	84	98 2	21 6 22 3	Left	7,720	95	4 06	After lumbar neurectomy			
				April 8	100	66	80	97 9	19 0 25 0	Left	5,700	95	3 00			
3	M	28	Spastic paraplegia	1926	98	60	76	98 2	13 0 23 0	Right	1,100	110	0 47	Before operation		
				February 16	98	64	72	98 1	-2 7 22 0	Right	600	110	0 27			
				February 17	100	60	70	97 0	-1 0 22 3	Right	3,000	110	1 36	After lumbar neurectomy		
				March 9	98	64	72	98 8	22 5	Right	740	83	0 44			
4	F	?	Raynaud's disease	1925	96	70	86	98 8	20 0 22 6	Right	2,200	83	1 32	Before operation		
				October 6	86	80	86	98 8	20 0 22 3	Right	1,760	83	1 06	After lumbar neurectomy		

			1926	February 4	110	60	72	98	6	-9	422	0	Right	960	115	0	41	Before operation		
				April 1	110	60	72	98	6	-9	422	0	Right	940	115	0	40	sympathetic		
				April 2	116	60	70	98	6	6	522	8	Right	1	380	115	0	64	After lumbar sympathectomy	
5	M	54	Buerger's disease	March 30	136	80	72	97	7	0	22	6	Right	1	200	104	0	57	At rest	
6	M	34	Buerger's disease	March 25	130	80	80	102	2	-2	0	22	3	Right	4	600	104	2	21	Following the injection of 35,000,000 typhoid bacilli

## DISCUSSION

Heat is eliminated from the body in different ways, through the excreta, expired air, evaporation of sweat, and by conduction and radiation from the skin. Vicordt (8) has estimated the relative values of these agencies in causing loss of heat as follows: urine and feces 1.8 per cent, expired air 10.7 per cent, evaporation from the skin 14.5 per cent, and radiation and conduction from the skin 73

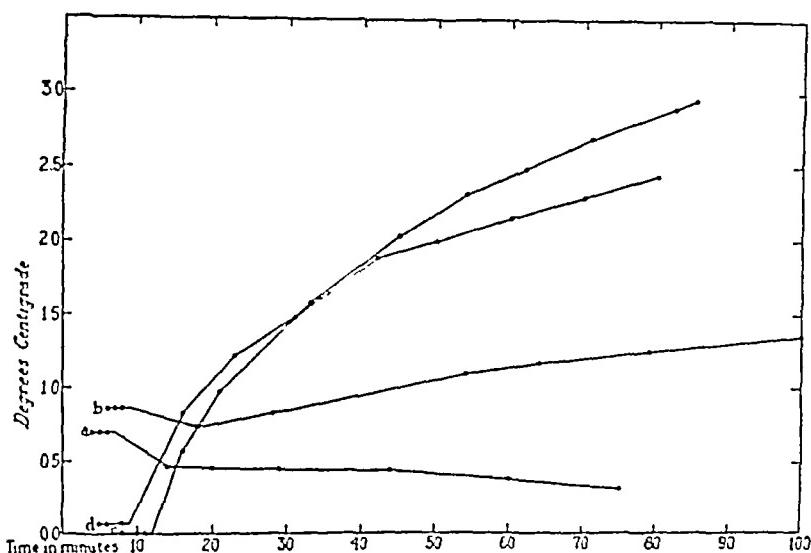


FIG. 3 INCREASED HEAT ELIMINATION IN CASE OF RAYNAUD'S DISEASE FOLLOWING LUMBAR SYMPATHETIC GANGLIENECTOMY AND PERIVASCULAR NEURECTOMY

Curves a and b Right foot, before operation

Curves c and d Right foot, 16 days after operation

per cent. In man, evaporation and radiation are largely the basis of heat regulation, the so-called physical regulation of loss of heat. The method of Stewart (6) for determining the rate of loss of heat, in which the extremity is immersed in water at a temperature lower than that of the skin, determines largely the loss of heat due to radiation and conduction rather than that due to evaporation. The source of the heat of radiation and conduction must be derived from

the surface circulation, the inherent tissue heat and the cellular or metabolic heat

Unfortunately, from the standpoint of quantitative study, the measurement of the heat output of the skin is complicated by vaso-motor effects. The vasomotor mechanism is delicately adjusted to the environmental temperature, and the circulation of the surface vessels, sweat gland activity and the blood concentration, according to Barbour (2), vary with changes in the environmental temperature. Many disturbing factors affecting the vasomotor nerves enter into this type of calorimetric work. The exposure of the feet to the air, the effects of plunging the foot into water of a lower temperature, the arrest of local perspiration, the contralateral vasomotor effect of the cooling of the opposite extremity, the compensatory after-effects of vasoconstriction illustrate some of the probable disturbances. As our data show, we have attempted to eliminate or control these disturbing agents so far as possible, but a definite limitation or absolute accuracy is impossible in this type of investigation. If an ideal type of experiment could be carried out in which the patient was adequately controlled for long periods of time under a constant environmental temperature, and perhaps an air-type of calorimeter was utilized and the various vasomotor disturbances were controlled or eliminated, data obtained would be much more accurate. For clinical purposes absolute accuracy is not required, as the range of loss of heat in both normal and pathologic subjects is wide. With a restricted environmental temperature comparative determinations show fairly consistent values by the Stewart-Kegerreis method.

Sheard's analyses of the heat curves apparently show that the temperature curve of the immersion water can be divided into two portions *a*, the rapidly rising, and *b*, the straight or linear. He has shown that these differences in the curves represent heat of different origin. As information was desired relative to blood flow, the second or linear segment of the curve was used as the basis of this measurement, as this portion represents the period of steady increase or transfer of heat from skin to water. If such an experiment were carried out for a sufficient length of time the curve would approximate a straight line parallel to the time axis when the bath and surface temperature become approximately equal.

Sheard's data seem to show that the loss of heat as measured in small calories cannot be interpreted in terms of volume flow of blood because of the lack of information on the conduction and radiation properties of the skin and subcutaneous tissue, the number and size of the surface vessels, and the rate of capillary flow. The difference in temperature between the surface blood and the immersion bath plays a part in determining the rate of loss of heat. The effects on the rate of heat elimination, of changing the conduction properties of the surface, can be verified by the simple expedient of covering the foot with an insulating material such as paraffin. Sharp decreases in the rate of heat elimination will then occur, with no evidence that the blood flow in the central part of the foot has been disturbed in the least. If the skin and subcutaneous tissues were susceptible of analysis as to their specific heat and conductivity constants, and the capillary factors were susceptible of measurement in the individual case, the values for loss of heat could then be transferred into terms of volume flow of blood. From the clinical aspect, the value of the method is not greatly impaired by this fact. A method has a clinical value and application when the data can be reduced to a quantitative unit and when the method can be so controlled that fairly accurate comparative data are obtainable. Many clinical procedures lack absolute value because of unevaluated factors which however are sufficiently constant to make comparative data of great significance. The estimation of blood pressure illustrates this point.

Our clinical studies on the value of the calorimetric method of investigating vascular diseases indicate clearly that under a fairly constant environmental temperature the range of heat elimination in normal subjects is fairly wide. Low rates of loss of heat are found in many apparently normal subjects with cold extremities whereas higher values are obtained in other normal subjects with warm extremities and the same environmental temperature. The so-called normal subject with cold extremities presents certain well-defined variations in the color and appearance of the skin of the acral areas. When coldness of the extremities becomes troublesome, the condition assumes a clinical interest and is designated acrocyanosis or acro-asphyxia. When pain and trophic disturbances ensue the condition is recognized clinically as Raynaud's disease. Studies of the surface capillaries by

the Lombard method indicate that the vascular disturbance varies only in degree in this large group and it is frequently difficult to state where abnormality begins (3). The main arteries of the limb seem normal and have the usual amplitude of pulsation as determined by the oscilloscope. There is no indication that the volume flow of blood entering the hand or foot is significantly diminished. This disturbance of a vasospastic nature seems to be confined largely to the peripheral arterioles, capillaries and venules of the skin, with a diminished flow of surface blood and thus a diminished loss of heat. There is some evidence that a portion of the arterial blood is shunted through the deeper arteriole channels. Thus it is clear why in many so-called normal subjects with patent arteries of the feet, the rates of heat elimination as measured in small calories of heat are as low as in subjects with obliterative vascular disease. The apparent paradox is explained by the fact that an adequate or marked collateral circulation is established in the latter cases. With gangrene of a small portion of the foot, such as a toe, the circulation for a small segment of tissue would be inadequate, but not in a degree to decrease markedly the loss of heat of the foot as a whole nor to influence significantly the calorimetric data. I have obtained low normal values of loss of heat in the foot with well-defined gangrene of one or more toes. From the standpoint of diagnosis the values of heat loss are not pathognomonic, although, generally speaking, normal subjects have higher rates of heat elimination than subjects with thrombo-angitis obliterans and lower rates than certain subjects with polycythemia vera. There is an intermediate range of values in which the groups overlap.

The vasomotor responses of the normal subject and of the subject with thrombo-angitis obliterans to elevations in the environmental temperature are strikingly different, as noted in our data. The normal subject shows marked vasomotor response with changes in the room temperature, these variations as measured in loss of heat reach several hundred per cent in magnitude. Repeated determinations on the pathologic subject indicate a more constant rate of loss of heat, and the vasomotor responses with changes in environmental temperature are less. The local circulation is theoretically maintained with vasodilatation at a high or maximal point in the extremity as a com-

pensatory effect of arterial obliteration. It was observed however that in some cases of thrombo-angitis obliterans vasodilatation was sufficient to be determined quantitatively by the foot calorimeter. As a result of this observation, a vascular test has been devised to determine the potential vasodilatation in response to increased systemic temperature in the different types of vascular disease, this will form the basis of a separate study. Information is thus obtained as to whether benefit would probably result from operative measures which would destroy, or produce interference with, the vasoconstrictor nerve paths.

The calorimetric method has been of great clinical value in comparative determinations to evaluate the effects of treatment. As shown in our data, under fairly constant environmental temperatures approximately similar data are obtained in repeated determinations on any given normal or pathologic subject. The effects of various therapeutic measures have been studied, and the constancy or variation in the rates of heat elimination have been used as a basis for determining their efficacy. The effects of the intravenous injection of sodium citrate and the oral administration of large quantities of Ringer's solution were found to be nil. No changes in the loss of heat were noted in cases of thrombo-angitis obliterans following the intravenous administration of radium chloride, although relief from pain was noted in 50 per cent of the cases. A series of patients with Raynaud's disease and thrombo-angitis obliterans were given intravenous injections of foreign protein (typhoid vaccine) with marked increases in the rate of heat elimination in the first group and in some of the latter group. The vasodilator effects following the removal of the second, third, and fourth lumbar sympathetic ganglia for the relief of muscle spasticity were demonstrated conclusively by the calorimetric method. This operation carried out in cases of Raynaud's disease resulted in the complete disappearance of all symptoms in the lower extremities and was associated with large increases in the rate of heat elimination. In four cases of thrombo-angitis obliterans the rates of heat elimination were increased following lumbar ganglionectomy, but not so markedly as in the cases of spastic paraplegia or of Raynaud's disease. Calorimetric determinations have been adopted as a routine procedure in the study of

localized peripheral vascular disease because of the great value of comparative studies on the rates of loss of heat.

The question naturally arises whether the calorimetric method gives more information than would the determination of surface temperature. I am of the opinion that multiple determinations of the skin temperature by an accurate technic would be of equal value. The calorimetric method has certain practical advantages since it gives the sum total of the surface heat eliminated from a large area of tissue in terms of calories lost during a unit space of time and for a unit area of surface. It gives convenient data for comparison and permits the establishment of certain approximately normal standards.

There are several disadvantages in the method. The average time for a determination is about one hour, and an exacting technic is necessary to obtain reliable data. When a room of constant temperature is not available the results are seriously interfered with by hot weather. During the excessively hot weather we have not attempted to use the method clinically.

#### SUMMARY

The calorimetric method of Stewart and of Kegerreis has been critically studied from the standpoint of its clinical value in the peripheral vascular diseases. It was found that the loss of heat of the extremities, as measured in small calories eliminated for each square inch of surface for each minute of time, could not be interpreted in terms of volume flow of blood. The normal range of heat elimination varies with changes in the environmental temperature. Under a restricted range of environmental temperature from 22° to 26°C., from 0.46 to 1.15 small calories of heat are eliminated for each square inch of surface area of the foot for each minute in normal subjects. In subjects with thrombo-angitis obliterans with obstruction of the main vessels of the feet from 0.27 to 1.0 calorie of heat is eliminated. The fluctuations in loss of heat from changes in the environmental temperature are much less in patients with obstructed arteries of the feet. From the diagnostic standpoint the method has a restricted value in the study of the vascular disturbances affecting the

extremities. For comparative studies under controlled conditions the calorimetric method is of great value as it makes possible the adequate evaluation of both medical and surgical treatment.

#### BIBLIOGRAPHY

- 1 Adson, A W, and Brown G E Jour Am Med Assn, 1925, lxxxiv, 1908-1910 Treatment of Raynaud's Disease by Lumbar Ramisection and Ganglionectomy and Perivascular Sympathetic Neurectomy of the Common Iliacs
- 2 Barbour, H G Physiol Rev, 1921, 1, 295-326 The Heat-Regulating Mechanism of the Body
- 3 Brown, G L Arch Int Med, 1925, xxv, 56-73 The Skin Capillaries in Raynaud's Disease
- 4 Brown, G F, and Adson, A W Am Jour Med Sc, 1925, clxx, 232-240 Calorimetric Studies of the Extremities Following Lumbar Sympathetic Ramisection and Ganglionectomy
- 5 Hewlett, A W and Van Zwietenburg, J G Heart, 1909-1910, 1, 87-97 The Rate of Blood Flow in the Arm
- 6 Stewart, G N Heart, 1911-1912, iii, 33-88 Studies on the Circulation in Man I The Measurement of the Blood-Flow in the Hands
- 7 Taylor, N B Jour Lab and Clin Med, 1922-1923, viii, 153-156 Observations upon the Blood Flow in Man II Estimation of the Blood Flow through the Hands in Clinical Cases
- 8 Vieordt, K H Quoted by Howell, W H Text-book of Physiology Philadelphia, W B Saunders Company, 9 ed, 1924 p 975

## STUDIES IN SCARLET FEVER

### I THE AMOUNT OF SCARLATINAL TOXIN IN THE BLOOD OF PATIENTS WITH SCARLET FEVER<sup>1</sup>

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#### INTRODUCTION

In an earlier paper by Trask and Blake (1), it was shown by studies in a small series of cases of scarlet fever, that a specific toxin is sometimes present in the blood during the acute stage of the disease. The presence of the toxin was demonstrated by its capacity to cause a local reaction in the skin of persons in whose serum there was no demonstrable scarlet fever antitoxin. The specificity of the reaction was shown by negative control tests in persons in whose serum scarlet fever antitoxin was present. The amount of toxin present was roughly estimated as +++, ++, + or - as judged by the size, intensity and duration of the local reaction resulting from the intracutaneous injection of 0.3 cc of patient's serum into susceptible persons. At the time the study was made no standard unit of scarlet fever toxin with which the toxin in the blood might be compared was available.

In 1925 Dick and Dick (2) described a unit for the toxin prepared from culture filtrates of *Streptococcus scarlatinae*. This unit is called a skin test dose and is defined by them as that amount of toxin which will give a positive skin reaction in those individuals who are susceptible to scarlet fever and a negative reaction in those who are not susceptible. An area of reddening, no matter how faint the color may be, that measures one centimeter or more in any diameter twenty-four hours after the intracutaneous injection of 0.1 cc of toxin constitutes a positive reaction.

<sup>1</sup> This work was done with the aid of the Goodhart Scarlet Fever Fund

Since immunity and susceptibility to scarlet fever are relative terms it is clear that no fixed amount of toxin will satisfy the definition, even were skin reactivity to the toxin the sole factor in determining susceptibility to scarlet fever. To make the definition more accurate it must be understood that the standard unit will give a barely positive reaction in the least susceptible of the presumably non-immune group, and negative reaction in the least immune of the presumably immune group. However, the skin test dose is at present the unit of toxin in general use and the strength of other toxins may be measured against this standard unit with some degree of accuracy.

It has seemed desirable, therefore, to determine how many units or skin test doses of toxin are present in the circulating blood of patients with scarlet fever in cases of varying degree of severity. This should give more precise knowledge concerning the degree of toxemia that may occur, than was obtained by the crude estimations in the previous study. The number of skin test doses of toxin present per cubic centimeter of serum has therefore been determined in twenty-five cases of scarlet fever and in each case the estimated clinical severity has been compared with the actual amount of toxin found.

#### EXPERIMENTAL METHODS

Serums from scarlet fever patients which had previously been shown to contain scarlet fever toxin were used. Four of these sera had produced a +++ reaction, ten a ++ reaction, and eleven a + reaction in susceptible test subjects.

The amount of toxin in the blood was determined by finding the smallest amount of serum which would give a positive reaction on intracutaneous injection in highly susceptible individuals. Then, by discovering the reactivity of the same individuals to the standard filtrate toxin, the toxin content of the blood serum could be calculated in terms of skin test dose units.

Serial dilutions of the serums to be titrated were made in 0.9 per cent saline. One-tenth cubic centimeter of each dilution was injected intracutaneously into human volunteers, susceptible and non-susceptible in each case. The former were two individuals, the most

reactive to the toxin among twenty susceptible people found in a study of forty-five people who gave a negative history of scarlet fever. The smallest amount of serum which caused in one or both of these individuals an erythema 1 cm. in any diameter twenty-four hours after injection was taken as the end point of the reaction. These two volunteers were then tested with serial dilutions of a standard filtrate toxin;<sup>2</sup> 1,  $\frac{1}{2}$ ,  $\frac{1}{4}$ ,  $\frac{1}{8}$ ,  $\frac{1}{16}$ , and  $\frac{1}{32}$  skin test doses being employed. It was found that a similar end point was obtained with  $\frac{1}{16}$  of a skin test dose of standard filtrates toxin. The number of skin test doses of toxin per cubic centimeter of the serum samples was calculated from this data. Thus if  $\frac{1}{16}$  cc. of serum was the smallest volume to give a positive reaction, there were per cubic centimeter 320 reacting doses for this test subject, who reacted similarly to  $\frac{1}{16}$  standard skin test dose. Hence in 1 cc. of the sample of blood serum there were 20 skin test doses of toxin.

The errors of this method of comparing the toxin contents of various samples and referring them to standard skin test doses are large and probably range between 100 and 400 per cent. However, the values obtained showed differences of a much greater order.

#### RESULTS

The results of the titrations are shown in table 1. It will be seen that the amount of toxin in the serum varied greatly in different patients, ranging from  $\frac{1}{4}$  of a skin test dose to 330 skin test doses per cubic centimeter.

It is also evident that the degree of toxemia in cases 2, 10, 11, 13, 14, 15, 22, 23, and 25 did not parallel the clinical severity of these cases. That this discrepancy exists is not surprising, since the estimate of clinical severity frequently depends upon a composite of the specific toxic and septic phases of scarlet fever, and at best, can be but a very rough estimate of the degree of the specific toxemia when septic processes are present.

The estimation of the toxin content of the blood samples from the observation of the extent of the local reaction to 0.3 cc. of serum, and recorded by the scheme of +++, ++, and +, corresponds roughly

<sup>2</sup> The standard toxin was that supplied by the Hygienic Laboratory of the United States Public Health Service, Washington, D. C.

with the titration of skin test doses. One may say that a +++ reaction indicates a much greater toxemia than a + and generally a greater toxemia than a ++. The comparison of the ++ and +

TABLE 1  
*Titration of toxin in blood serum of scarlet fever patients*

Case	Clinical severity	Blood serum of patients	
		Skin reaction in test subject to 0.3 cc. of serum	Skin test doses per cubic centimeter
1	Extreme	+++	330
2	Mild	+++	120
3	Severe	+++	40
4	Severe	+++	40
5	Severe	++	25
6	Severe	++	10
7	Moderate	++	5
8	Moderate	++	5
9	Moderate	++	5
10	Extreme	++	2½
11	Severe	++	1½
12	Moderate	++	1½
13	Extreme	++	½
14	Extreme	++	½
15	Mild	+	5
16	Moderate	+	2½
17	Mild	+	2½
18	Moderate	+	½
19	Moderate	+	½
20	Moderate	+	½
21	Mild	+	½
22	Severe	+	½
23	Extreme	+	½
24	Moderate	+	½
25	Severe	+	½

+++ indicates strongly positive reaction, 50 to 70 mm. in diameter, bright red, moderately indurated and tender, of 3 to 4 days' duration followed by pigmentation and desquamation, ++, positive reaction, 30 to 50 mm. in diameter, bright red, with slight induration, of 2 to 3 days' duration, followed by moderate pigmentation and occasionally slight desquamation, +, moderately positive reaction, 20 to 35 mm. in diameter, red, no induration, of 1 to 2 days' duration, with slight pigmentation and no desquamation.

reactions with the titrated values shows that it is difficult to judge the strength of toxin in a given sample of serum from the observation of a single moderate reaction in a highly susceptible volunteer.

## DISCUSSION

The titration of the amount of scarlet fever toxin in the blood serum in a small series of cases has shown that the toxin content varies through extremely wide limits which are more than a hundred times the experimental error of the methods. Occasionally, a very low toxin content has been found in the serum from a patient extremely sick. When the importance of the septic phase of scarlet fever and the probable variations in the duration of the specific toxemia are taken into consideration this is not difficult to understand. It is, however, quite surprising to find a large amount of toxin in the serum of an apparently mild case, as shown in case 2. This would lead one to suspect that the general susceptibility of individuals to the toxin may depend on more factors than the mere absence of specific antitoxin. This appears to be the case in laboratory animals, which are highly refractory to even large amounts of toxin in culture filtrates of *Streptococcus scarlatinae*, and yet in their blood serum no specific scarlet fever antitoxin is found.

The wide and often unpredictable variations in the amounts of toxin in the various samples studied, indicates that a large excess of antitoxin should be used for therapeutic purposes to obtain consistently satisfactory results.

## SUMMARY AND CONCLUSIONS

1 The amount of scarlet fever toxin found in the blood of scarlet fever patients during the acute stage of the disease varies between very wide limits.

2 The size and intensity of the local reaction caused by 0.3 cc. of serum from scarlet fever patients in the skin of susceptible persons provides a rough but fairly satisfactory measure of the amount of toxin in the serum.

3 Clinical estimation of the degree of toxemia in individual patients with scarlet fever is subject to a considerable error.

4 Because of the difficulty of estimating the actual degree of toxemia by clinical observation, a generous excess of antitoxin should be used in the treatment of scarlet fever if the best results are to be obtained.

## BIBLIOGRAPHY

- 1 Frask, J D , and Blake, F G Jour Exp Med , 1924, xl, 381 Observations on the Presence of a Toxic Substance in the Blood and Urine of Patients with Scarlet Fever
- 2 Dick, G H , and Dick, G L Jour Amer Med Assoc , 1925, lxxxiv, 1477 Results with the Skin Test for Susceptibility to Scarlet Fever

## STUDIES IN SCARLET FEVER

### II THE RELATION OF THE SPECIFIC TOXEMIA OF SCARLET FEVER TO THE COURSE OF THE DISEASE<sup>1</sup>

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#### INTRODUCTION

It has been shown in previous papers that a specific toxin is present in the blood of patients with scarlet fever during the early acute stage of the disease (1) and that the amount of toxin present varies within wide limits in different patients (2). The present communication deals with a further study of the specific toxemia of scarlet fever made in an effort to correlate the degree and course of the toxemia with the clinical course of the disease. It is based on the data obtained from the study of 132 of the 254 cases of scarlet fever admitted to the Medical and Pediatric Services of the New Haven Hospital from January, 1924, to June, 1925.

The conception of a disease formed on the basis of the compilation of single observations on many cases is less satisfactory than one resulting from the more detailed study of selected cases. However, this latter method was not open to us because all the patients more than moderately sick, and almost all those seen early in the disease were treated with scarlet fever antitoxin. Since efficient treatment promptly neutralizes the toxin in the blood (3) (4) (5), there was available for the study of the toxin in the great majority of the cases, only the blood taken immediately before treatment. In all, one hundred and forty-three serum samples were obtained from one hundred and thirty two patients. From four untreated cases there were repeated bleedings and from three cases repeated bleedings.

<sup>1</sup> This work was done with the aid of the Goodhart Scarlet Fever Fund

before treatment. The presence or absence of demonstrable scarlet fever toxin in the blood samples was determined and the amount of toxin present was roughly estimated. These observations were then analyzed with reference to the day of the disease on which the sample was obtained, the estimated clinical severity of the case, the presence of rash, and finally the presence of septic complications. Thus a composite picture was constructed, based, for the most part, on single observations in as many patients as could be studied.

#### EXPERIMENTAL

*Methods.* The serum from the blood samples was stored in the ice-chest without preservative. The toxin in the serum was demonstrated by injecting 0.3 cc intracutaneously into known susceptible and non-susceptible human volunteers, in whose blood the presence or absence of scarlet fever antitoxin had previously been determined. The usual site of inoculation was the flexor surface of the upper and fore arms. Readings were taken at twenty-four-hour intervals. The test was considered negative if there was no definite local erythema noted in the susceptible volunteer. The test was considered positive when there was in the susceptible volunteer a local erythema 1.5 cm or more in any diameter, at twenty-four hours. The positive reactions varied considerably in size and intensity and consequently were divided arbitrarily into +, ++ and +++ reactions as follows: + reactions indicate a moderate erythema at twenty-four hours, 1.5 to 3.5 cm in diameter, which was frequently followed by slight pigmentation; ++ reactions consisted of a marked erythema 3.5 to 5.0 cm in diameter which generally persisted two to three days, was associated with slight induration and tenderness, and was followed by moderate pigmentation and occasionally slight desquamation; +++ reactions indicate an intense erythema 5.0 to 9.0 cm in diameter, of three to four days duration, moderate induration and tenderness, followed by pigmentation and desquamation.<sup>2</sup>

<sup>2</sup> With the serums causing a +++ reaction, the non-susceptible volunteers frequently gave a + reaction. In all other cases the non-susceptible controls remained negative. It was noted that among the susceptible volunteers there was considerable variation in reaction, but that this variation followed a regular order in that those who gave the strongest reaction to any sample, also reacted most to

Scarlet fever antitoxin was demonstrated by means of the Schultz Charlton phenomenon (6). Of the serum to be tested 0.5 cc was inoculated intracutaneously in a scarlet fever patient. A site was chosen where the rash was bright red and diffuse. Readings were taken eighteen hours after inoculation and at twenty-four hour intervals thereafter. If definite local blanching of the rash was noted at any reading the test was considered to show the presence of scarlet fever antitoxin. If no blanching was seen at any time and there were satisfactory positive controls the test was considered negative. The test for antitoxin was made with sera from all the human volunteer test subjects, with samples of serum from all the late cases, and with sera from many of the early cases of scarlet fever. All the tests, whether for toxin or antitoxin, were made on samples of serum taken before the administration of scarlet fever antitoxin for therapeutic purposes.

An estimate of the clinical severity of the disease was made for each patient and recorded as in one of four groups, extreme, severe, moderate, and mild. The extreme group comprised those patients in whom the immediate prognosis as to recovery was doubtful. They all showed the appearance of profound intoxication, and also suffered from some more or less severe septic process. In the severe group were placed those who appeared very sick and usually suffered from some septic process, generally less severe than the septic process occurring in the former group. The moderate cases appeared moderately sick. Septic processes were generally less frequent and less severe than in the preceding groups. The mild cases varied from those showing scarcely any evidence of disease except fever, sore throat and rash to those who seemed less than moderately sick. In some of the cases of this group, however, considerable purulent rhinopharyngitis was present. The fever corresponded roughly to the estimated

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all samples, and frequently gave + reactions with serums to which the less reactive remained negative. At least one highly susceptible person was used in each test, and it was the reaction of this individual which determined the final reading for the specimen. Certain serums in either or both the test and control volunteers gave an immediate wheal and erythema reaction similar to the allergic skin reactions seen in hay fever and asthma. This reaction had no relation to the presence of scarlet fever toxin.

clinical severity. The rash was studied and recorded as bright, fading or faded, or absent. A special note was made in each instance as to the presence and character of septic processes or complications. The first day of the disease was considered as that on which the initial symptoms of the onset appeared. The days were reckoned

TABLE I

*Incidence and degree of specific toxemia in 121 cases of scarlet fever with rash present*

Clinical severity	Cutaneous reaction of susceptible volunteers to toxin in blood of patients	Day of disease								Totals		
		1	2	3	4	5	6	7	8	In groups	Positive	Negative
Extreme	+++			2				1		3	11	
	++		3		1					6		
	+		1							2		
	-					1				1		1
Severe	+++		1	1		1				3	20	
	++		4	3	2					9		
	+		2	2	3	1				8		
	-	1		2						3		3
Moderate	+++									0	31	
	++		1	4	3	1	1	1		11		
	+		9	4	2	3	1			20		
	-	2	3	2						7		7
Mild	+++			1		1				2	28	
	++		3	1		2	1			7		
	+	1	3	9	5	1				19		
	-	4	6	11	7	3				31		31
Totals	+++		1	4		2		1		8	90	
	++		11	8	6	3	3	2	1	33		
	+	1	15	15	10	5	1	1	1	49		
	-	7	9	15	7	4				42		42
Per cent positive		13	75	64	70	77	100	100	100			

as periods of twenty-four hours from hour of onset in all those patients admitted up to the fifth day. For those admitted on the fifth day or later, time was reckoned as calendar days.

*Results.* In table 1 are listed the results of tests for toxin in 132 samples of blood serum from 121 scarlet fever patients. These

represent all the samples collected from patients who still seemed sick up to the ninth day of the disease, and in whom the rash was still present. The cases are grouped according to the day of disease on which the sample of blood was obtained and according to the estimated clinical severity. The toxin is recorded as not demonstrable (-) or, if demonstrable, as +, ++, or +++, depending upon the size, intensity, and duration of the cutaneous reaction of the sus-

TABLE 2  
*Eleven cases of scarlet fever with fading or faded rash*

Case	Clinical severity	Rash	Complications	Blood			
				Day	Toxin	Antitoxin	Culture
P. H.	Moderate	Fading	Jaundice Septic tonsillitis	7	+	-	-
F. S.	Mild	Fading	Cervical adenitis	7	+	-	-
S. S.	Mild	Faded	Cervical adenitis	8	+	-	-
C. S.	Severe	Faded	Otitis media Mastoiditis Cervical adenitis	9	+	-	-
A. S.	Mild	Faded	Purulent rhinitis. Cervical adenitis	10	+	-	-
M. N.	Severe	Faded	Purulent rhinitis Otitis media Cervical adenitis	13	+	-	-
E. D.	Extreme	Faded	Septicemia Purulent arthritis	14	-	+	+†
G. G.	Extreme	Faded	Otitis media Broncho pneumonia	14	-	+	-
R. H.	Mild	Faded	Seventeenth day cervical adenitis	19	-	+	0
H.	Mild	Faded	Nineteenth day cervical adenitis	21	-	-	0
V. H.	Mild	Faded	Twentieth day cervical adenitis	21	-	-	0

\* No reaction for toxin in this group was stronger than +

† Pure culture *Streptococcus hemolyticus*

ceptible volunteer. The numbers in the columns represent the total numbers of samples in each group.

In table 2 are shown all cases studied in whom the rash was fading or had faded and who still seemed sick. In these the presence of septic complications is noted. The results of the tests for the presence of toxin and antitoxin in the serum samples is recorded, and also the results of blood cultures when these were made.

Analysis of the data in table 1 shows that there is a striking increase

in the incidence of demonstrable specific toxemia during the first two days of the disease, then a slight decrease on the third day, which is followed by a gradual increase to reach 100 per cent on the sixth day. The cases on the first and second days represent a relatively homogeneous group of patients who for the most part had not as yet reached the peak of the disease. Those of later days represent a less homogeneous group of patients, some of whom had begun to recover, some of whom were stationary and some of whom were becoming sicker. It seems probable, therefore, that the decreased incidence of the third and fourth days is due to the inclusion in the group of some patients who were beginning to recover and in whom the specific toxemia was abating.

The high incidence on the later days of the first week does not mean that the course of the specific toxemia is usually so extended, but is undoubtedly due to the fact that only those who were still sick were included in the study. That this is so is illustrated by the course of the toxemia in N. G., a moderate case of scarlet fever without complications, in whom the temperature reached normal on the sixth day. Repeated bleedings were made with the following results: Second day +, third day ++, fifth day +, sixth day -.

The duration of the specific toxemia in patients remaining sick after the first week has been studied in only ten cases, nine of which are shown in table 2, the tenth being included in table 1 because the rash was still present. From so small a number it is wise merely to say that the latest record of demonstrable specific toxemia was on the thirteenth day and that the earliest record of the natural appearance of demonstrable antitoxin in cases that remained sick was the fourteenth day. In the cases in which the rash was still present the relation of the degree of clinical severity to the incidence of demonstrable toxemia and to the amount of toxin in the blood was close. This is shown in the last column of table 1. This relation did not hold in the cases in which the rash was fading or had faded, as is shown in table 2. In none of these cases did the test for toxin show a stronger reaction than +, while in the two extreme cases there was no toxin at all.

## RELATION OF SPECIFIC TOXEMIA TO SEPSIS

In the data presented above all the cases have been grouped primarily as to whether they were in the exanthematous or post-exanthematous stage, secondarily according to the day of the disease and total clinical severity, irrespective of the presence or absence of septic complications. Since it is well recognized that as the disease progresses septic processes play an increasingly important rôle as regards the total clinical severity, it has seemed desirable to study the relation of sepsis to the incidence and degree of specific toxemia.

Sepsis is used here to mean the local or distant invasion of the tissues of the host by *Streptococcus scarlatinae*. In the cases studied the majority of the septic complications were apparently due to the direct spread of the primary infection to the mucous membrane of adjacent structures, such as nose, sinuses and pharynx. Not infrequently the infection extended to the middle ear, less frequently to the mastoid cells, and in one case to the meninges. There was also in some cases invasion of the deeper tissues and lymph nodes. In these places suppuration occasionally followed. There was one case of postpartum scarlet fever with parametritis and general peritonitis.

The presence of a positive blood culture was an uncommon finding, occurring in but one of the fifty two cases in which blood cultures were taken. It is recorded under case L D, table 2. The most frequent single complication observed was a purulent rhinopharyngitis. The mere presence of a slight amount of exudate on the tonsils was not considered as evidence of invasion of the body tissues. When there was definite ulceration of the tonsils, however, the case was included in the septic group.

Based on ordinary clinical examination an attempt was made to evaluate the importance of any septic process which was present at the time the blood samples were obtained. The presence of one or more of the following conditions was considered to constitute an extreme degree of sepsis and classified as +++ sepsis, septicemia, septic arthritis, meningitis, acute purulent mastoiditis, ulcerative stomatitis, suppurative cervical adenitis, and parametritis. The presence of one or more of the following conditions was considered to constitute a moderate degree of sepsis and was classified as ++.

sepsis severe purulent rhinopharyngitis with or without sinusitis, purulent otitis media, ulcerative tonsillitis, severe non-suppurative cervical adenitis and peritonsillar abscess. The presence of one or more of the following was considered to constitute a mild degree of sepsis and was classified as + sepsis moderate or mild purulent rhinopharyngitis, with or without sinusitis, and moderate non-

TABLE 3

*Specific toxemia of scarlet fever in respect to degree of sepsis and day of disease in 121 cases with rash present*

Degree of sepsis	Cutaneous reaction of susceptible volunteers to toxin in blood of patients	Day of disease								Totals		
		1	2	3	4	5	6	7	8	In groups	Positive	Negative
+++	+++			1				1		2	3	
	++									1		
	+									0		
	-									0		0
++	+++		1	2	2	1				4	22	
	++	5	2	2	1	1	1	1		11		
	+	1	1	2	1	1	1	1		7		
	-				1					1		1
+	+++		1		1					2	33	
	++	2	2	4	2	1				11		
	+	6	5	6	2	1				20		
	-	2	5	4	3					14		14
-	+++									0	32	
	++	4	4		1					10		
	+	1	8	9	2	2				22		
	-	5	4	11	4	3				27		27

suppurative cervical adenitis. Such a scheme is subject to much error but was as satisfactory as the conditions permitted, and was probably not any more inaccurate than the method used to judge the degree of specific toxemia.

The relations between the septic and toxic factors in the disease are presented in table 3 and figure 1.

Table 3 records observations on the same group of cases as those

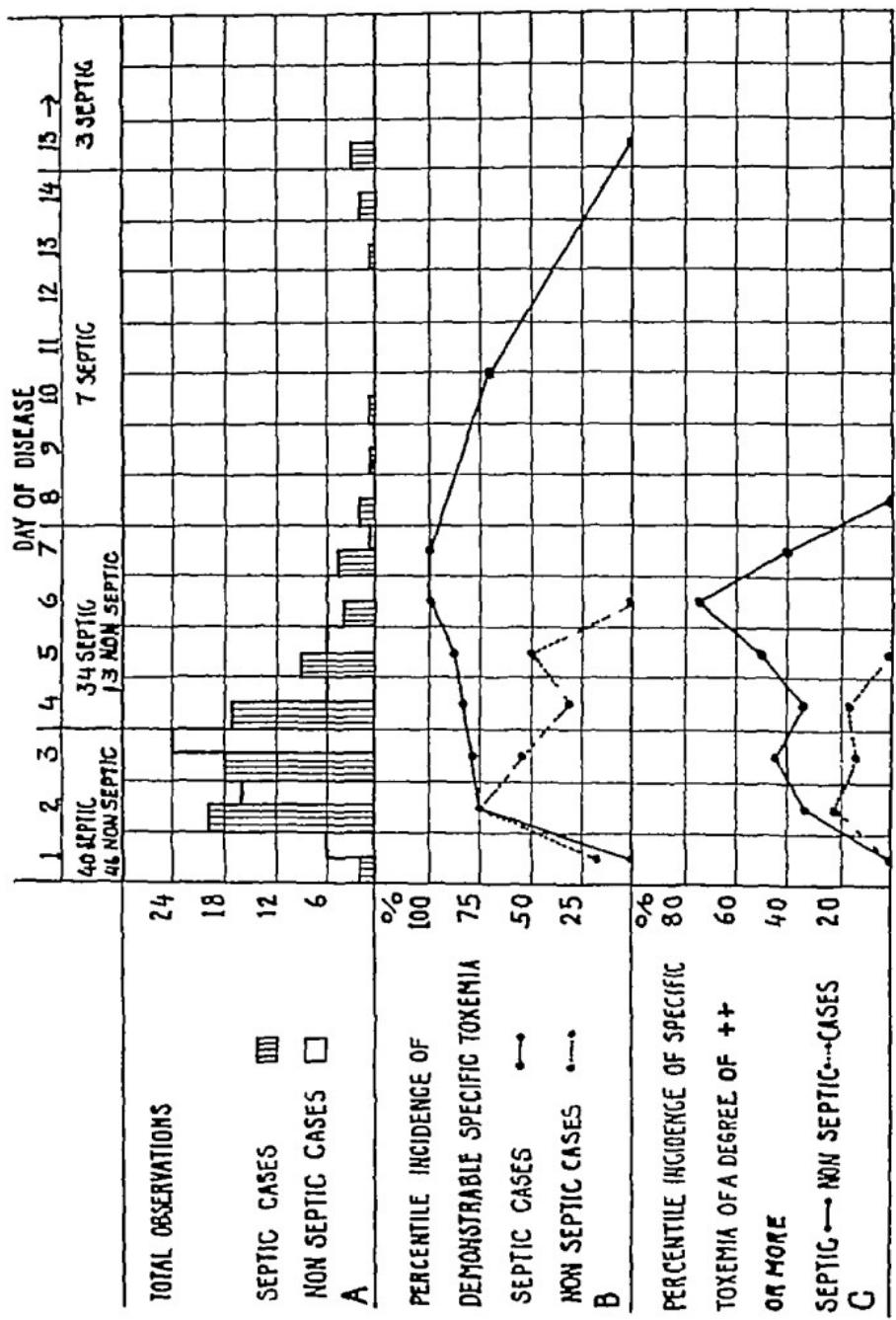


FIG 1

in table 1, 1 c, all cases in which the rash was still present at the time the blood was collected for the determination of the presence of toxin

Comparison of the columns of totals on the right side of tables 1 and 3 shows that while the incidence and degree of specific toxemia agree quite closely both with the estimation of total clinical severity and with the severity of the sepsis, the degree of specific toxemia agrees more closely with the estimated severity of sepsis than with the estimated total clinical severity. That this relationship does not hold after the rash has faded or, in terms of duration, after the first week of the disease, has already been pointed out in connection with the group of cases shown in table 2.

The relationship throughout the course of the disease, irrespective of the presence or absence of the rash is shown graphically in figure 1 in which all cases in the study are included.

In figure 1 (a) it will be seen that the ratio of septic to non-septic cases increases rapidly after the third day of the disease among patients who remain sick. In this particular group of patients the ratio was 40 46 for the first three days, 34 13 for the fourth to seventh days, and 10 0 after the first week. All the cases in the first week, with two exceptions on the seventh day, had a bright rash. In all cases after the first week, with one exception on the eighth day, the rash had faded.

From figure 1 (b) it will be seen that the percentile incidence of demonstrable specific toxemia rises abruptly to 75 per cent on the second day of the disease in both the septic and non-septic groups but that after this point there is a marked divergence. The incidence for the septic group continues upward gradually to reach 100 per cent on the sixth day, and maintains the level to the thirteenth day. On the fourteenth day it falls abruptly to zero. This is not shown by the chart, however, since, as there were but seven cases in the second week, they were all grouped together and plotted on one point at the middle of this period. The incidence for the non-septic group falls after the second day and reaches zero on the sixth day. On the seventh day occurred the last non-septic case and in this instance specific toxemia was demonstrated. But this case has been omitted in plotting the curve as it would give an undue weight to a single exceptional finding.

This apparent exception emphasizes the fact that the relations between the various features of the disease depend on more variable factors than have been considered. In this case it was impossible to fix the date of onset accurately as the onset was gradual during the course of common head cold. If the date of onset was reckoned as early as possible the serum was obtained on the seventh day. However this would put the appearance of the rash on the fourth day, which is two days later than usual. Therefore it seems likely that onset was dated too early.

It is desirable to know the changes in the degree of specific toxemia during the disease. If table 1 is analyzed for the incidence of +++ reactions, and if the cases are taken in periods of two days to obtain larger groups one finds the per cent of cases showing +++ reactions to be as follows for the first and second day 2, for the third and fourth 6, for the fifth and sixth 11, and for the seventh and eighth 20. After this time no reactions stronger than + were found.

To establish a curve for degree of toxemia on the basis of more cases, and to correlate this with the presence or absence of septic complications, figure 1 (c) was constructed. This shows the percentile incidence, for each day, of cases with a ++ or +++ specific toxemia in the septic and non-septic groups. The non septic case on the seventh day which was omitted from figure 1 (b) had a ++ reaction and is also omitted from figure 1 (c) for the reason given above. The difference between the two groups is obvious. The specific toxemia is greater throughout in the septic cases. The peak for the non septic cases is on the second day, but for the septic cases is on the sixth day. The figure also shows that the rather high incidence for the septic group during the second week is associated with a low degree of specific toxemia. This emphasizes that only while the rash is present does the intensity of the toxemia vary with the incidence of septic complications.

#### DISCUSSION

This study has been concerned with the most common types of scarlet fever, namely, those of various grades of clinical severity, with and without septic complications. In an attempt to relate the demonstrable specific toxemia to the clinical features of the dis-

case a composite picture has been constructed from all observations. This procedure probably has obscured some information which might be brought to light in a detailed investigation of cases untreated with antitoxin.

Determinations of the presence of scarlet fever toxin in the blood serum of patients and rough estimations of the toxin content have been made and the results have been discussed in relation to certain clinical features of scarlet fever. These features are the duration of the disease, the presence or absence of the rash, the estimated total clinical severity and the presence and apparent severity of septic complications.

The present conception of scarlet fever holds that the characteristics of the disease by which it is differentiated from other hemolytic streptococcus infections depend in large part on the presence of scarlet fever toxin in the blood stream of the patient and its action on the body. Accordingly one would expect that the rash, which is the most striking sign of scarlet fever, would in some way run parallel with the specific toxemia. The results given above suggest that the duration of the specific toxemia is measured by the duration of the rash. In severe septic cases remaining sick into the second week of the disease, the rash is usually pigmented and consequently it is often difficult to decide whether it is fading or faded. In this group the specific toxemia was found to be of low degree. In the later septic cases when the rash was completely faded there was found, even in two extremely sick patients, not only no toxin but an actual excess of autogenous scarlet fever antitoxin.

The data accumulated in this study provide definite indications with respect to the therapeutic use of scarlet fever antitoxin. In the first place it is clear that antitoxin should be administered as early as possible in the disease in order to check the toxemia during its period of increase. In the second place it is obvious that patients with incipient septic processes early in the disease are potentially much sicker than those without septic processes. Consequently these patients should receive more antitoxin, even though they may not appear more severely ill at the moment. In the third place it is evident the largest amounts of antitoxin are required in those cases with severe septic processes in whom the rash is still bright. Finally

it is apparent that in late cases with faded rash little or no benefit may reasonably be expected from antitoxin therapy.

The observations indicate that *Streptococcus scarlatinae* may have at least two quite different methods of attack, and that the defensive mechanisms of the host against these may be dissociated. Variable combinations of these factors result in different clinical pictures of the disease.

#### SUMMARY AND CONCLUSIONS

- 1 The specific toxemia of scarlet fever is a self limited phase of the disease.
- 2 The duration of the specific toxemia of scarlet fever parallels the duration of the rash.
- 3 The degree of specific toxemia of scarlet fever, while the rash is present, depends largely on the presence and severity of septic complications.
- 4 When the rash has faded the specific toxemia of scarlet fever is of low degree or has terminated, even though severe septic complications continue.

#### BIBLIOGRAPHY

- 1 Trask, J. D., and Blake, F. G. *Jour. Exp. Med.*, 1924, xl, 381. Observations on the Presence of a Toxic Substance in the Blood and Urine of Patients with Scarlet Fever.
- 2 Trask, J. D. *Jour. Clin. Invest.*, 1926, viii, 391. Studies in Scarlet Fever. The Amount of Scarlatinal Toxin in the Blood of Patients with Scarlet Fever.
- 3 Blake, F. G. *Trans. Assoc. Amer. Physicians*, 1924, xxxix, 141. Observations on the Treatment of Scarlet Fever with Scarlatinal Antistreptococcic Serum.
- 4 Blake, F. G., and Trask, J. D. *Trans. Assoc. Amer. Physicians*, 1925, xl, 7. Further Observations on the Treatment of Scarlet Fever with Scarlatinal Antitoxin.
- 5 Blake, F. G., and Trask, J. D. *Boston Med. and Surg. Jour.*, 1925, cxcmi, 659. The Treatment of Scarlet Fever with Antitoxin.
- 6 Mair, W. *Lancet*, 1923, ii, 1390. An Immunity Reaction in Scarlet Fever.



## STUDIES IN SCARLET FEVER

### III INFECTIONS WITH STREPTOCOCCUS SCARLATINAE IN PERSONS WITH SCARLATINAL ANTITOXIC IMMUNITY<sup>1</sup>

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#### INTRODUCTION

For a number of years writers have noted that cases of sore throat without a rash may occur among persons who have been exposed to scarlet fever. Hebra (1866) speaks of the "Scarlatina sine exanthemate." He says the use of this expression can be justified only in such cases as the following,—"several persons residing in the same locality and exposed to similar epidemic influences, fall ill at the same time, some of them present, in a well marked form, all the symptoms of scarlatine, others suffer merely from fever and an affection of the throat, there being in these patients no efflorescence, nor, at a later period, any desquamation."

Thomas (1875) mentions cases of irregular scarlet fever in which the chief symptoms are angina, slight fever, malaise lasting but a few days. He designates these cases as "angina scarlatinosa" and "febris scarlatinosa sine exanthemate sive sine scarlatina." He says "every throat affection during a scarlet fever epidemic is suspicious."

Leichtenstern (1882) in describing the scarlet fever epidemic in Köln mentions cases of scarlet fever without a rash which later developed severe nephritis.

Waring (1921) gives a report of an epidemic of septic sore throat which occurred in an army hospital. When the epidemic was at its

<sup>1</sup> This work was done with the aid of the Goodhart Scarlet Fever Fund.

<sup>2</sup> This paper is in part a thesis presented to the Yale University, School of Medicine in candidacy for the degree of Doctor of Medicine.

height an outbreak of scarlet fever suddenly appeared in the hospital. There was not a single case of scarlet fever within a radius of one hundred miles. Ward A 2 stood at the head of the list of wards in the number of cases of septic sore throat and also of cases of scarlet fever. An effort was made to discharge patients with septic sore throat due to hemolytic streptococci when the throats were free from these organisms. The scarlet fever outbreak then subsided.

During the last few years a number of writers have cultivated from the throats of persons without scarlet fever streptococci having the same characteristics as those of *Streptococcus scarlatinae*. Bliss (1920) found that three of seventeen strains of *Streptococcus hemolyticus* of non-scarlatinal origin were specifically agglutinated by antiserums prepared by immunizing animals with scarlatinal streptococci. He points out that all three strains were obtained from persons who had been in contact with scarlet fever.

Williams (1925) found that two strains from sources other than scarlet fever fell in the group of scarlatinal strains. One was from a wound and the other from a case of endocarditis. Two other strains, one from a case osteomyelitis and one from a case of bronchitis produced toxic filtrates neutralizable by convalescent scarlet fever serum. She also found that of fifty-six excised tonsils fourteen contained hemolytic streptococci, six of which produced toxic filtrates neutralized by convalescent scarlet fever serum.

Stevens (1926a, b) quotes the history of six cases of acute throat infections caused by *Streptococcus scarlatinae*. All these cases had been in contact with scarlet fever and they were apparently the source of infection for other cases of scarlet fever.

Rosenow (1926) reported five cases of scarlatinal infection, with positive precipitin reaction but with no rash. Two of the cases had previously had scarlet fever. In all five cases the Dick test was negative.

Stevens and Dochez (1926) found that five of seventeen strains of *Streptococcus hemolyticus* which they obtained from cases of acute pharyngitis during an epidemic of scarlet fever and angina, showed the agglutination and the toxin producing properties of *Streptococcus scarlatinae*. They also found that these cases of pharyngitis occurred in individuals with a negative Dick reaction. They point out that

the Dick test is not a reliable index of immunity to throat infections with *Streptococcus scarlatinae*

The earlier clinical observations cited above suggest the probability that infections with *Streptococcus scarlatinae* may occur not infrequently without the infected person developing those signs and symptoms upon which the clinical diagnosis of scarlet fever depends. The more recent bacteriological studies, especially those of Bliss, Stevens, and Stevens and Dochez, demonstrate that these infections actually do occur. It has become, therefore, a matter of considerable epidemiological importance to determine the frequency of occurrence of pyogenic infections with *Streptococcus scarlatinae* in individuals without scarlet fever. It is equally important for the elucidation of the pathology and immunology of infections with *Streptococcus scarlatinae* to discover, if possible, under what circumstances scarlatinal streptococcus infection may occur without the infection causing the specific clinical features of scarlet fever.

The present study was, undertaken therefore, in order to determine (1) how frequently pyogenic infections with *Streptococcus scarlatinae* occur without the characteristic rash of scarlet fever accompanying the infection, (2) whether there is any relation between the occurrence of these infections and known exposure to cases of scarlet fever, (3) whether the persons so infected are protected against the development of the specific toxic phase of scarlet fever by already possessing an antitoxic immunity, and (4) whether the possession of an antitoxic immunity to scarlet fever also provides an immunity to pyogenic tissue infections with *Streptococcus scarlatinae*.

#### SOURCE OF MATERIAL

Twenty-one strains of *Streptococcus hemolyticus* were obtained in cultures from twenty one patients suffering from various acute infections. The cases were not chosen from a selected group. All cultures coming to the laboratory which showed *Streptococcus hemolyticus*, and were not from scarlet fever patients, were studied. The majority of the strains were isolated from throat cultures from patients with tonsillitis, pharyngitis or sinusitis. There were four exceptions—one was obtained from the sputum from a case of pneumonia, one from the blood from a case of septicemia, one from a pleural exudate and the fourth from a discharging ear.

## METHODS

The method used for the identification of *Streptococcus scarlatinae* was that described by Dick and Dick (1925) with some necessary modifications. The organisms were isolated in pure culture on blood agar plates and a tube of broth containing 1 per cent of defibrinated rabbit's blood was inoculated from a single colony. The blood broth culture was incubated for four days. It was then filtered through a Berkesfeld filter and the sterility of the filtrate was determined. The filtrate was then diluted 1:100, 1:500, and 1:1000 with sterile salt solution. One-tenth of a cubic centimeter of each of these three dilutions was inoculated intracutaneously into the arm of an individual having a positive Dick test. The tests were read twenty-four hours after injection. A resulting area of erythema was tentatively considered to indicate that toxin was present in the filtrate. A positive reaction having a diameter of approximately one centimeter was recorded as a + reaction, between one and two centimeters as a ++ reaction, and over two centimeters as a +++ reaction. Faint reactions under one centimeter were recorded as a ± reaction of doubtful significance. One-tenth of a cubic centimeter of the dilution giving a ++ reaction was selected as a suitable skin test dose for subsequent tests. These consisted of control tests in Dick negative individuals and neutralization tests with blanching and non-blanching human serums.

Neutralization tests were performed as follows: a dilution of the filtrate as made up so that 0.5 cc contained 10 skin test doses of toxin. This was mixed with equal parts of blanching and non-blanching human serums, five blanching and five non-blanching serums being used with each filtrate. A control of the activity of the filtrate was made by mixing 0.5 cc of the same dilution of filtrate with an equal part of sterile saline. All tubes were incubated for one hour at 37°C. One-tenth of a cubic centimeter of each mixture was then inoculated intracutaneously into the arm of an individual having a positive Dick test. Neutralization of the toxic action by the blanching serums, provided the non-blanching serums failed to neutralize, was considered satisfactory evidence that the strain of hemolytic streptococcus from which the filtrate was prepared was *Streptococcus scarlatinae*.

## EXPERIMENTAL

*Experiment 1* Diluted filtrates from twenty one strains of *Streptococcus hemolyticus* were injected intracutaneously in a subject susceptible to scarlatinal toxin as previously determined by a Dick

TABLE 1  
*Determination of the presence of soluble toxin in the filtrates from 21 cultures of Streptococcus hemolyticus*

Filtrate number	Skin reactions in Dick positive subjects			Reactions to 1 skin test dose of filtrates	
	Dilution of filtrate			Dick-positive subject	Dick-negative subject
	1 100	1 500	1 1000		
1	++	+	±	+	-
2	++	+	±	+	-
3	++	++	+	+	-
4	+++	++	+	+	-
5	++	+	±	+	-
6		+++	++	+	-
7	+++	++	+	+	-
8	+++	++	+	+	-
9	++	+	±	+	-
10		+++	++	+	-
11	++	+	±	+	-
12	++	+	±	+	-
13	+++	++	+	+	-
14	++	+	+	+	-
15	+++	++	+	+	-
16	++	±	-	+	-
17	++	+	±	+	-
18	++	+	±	+	-
19	++	±	-	+	-
20	++	+	±	+	-
21	++	+	±	+	-

+++ = local erythema more than 2 cm. in diameter ++ = local erythema between 1 and 2 cm. in diameter + = local erythema approximately 1 cm. in diameter ± = faint reaction less than 1 cm. in all diameters. - = no reaction. Readings were made 24 hours after injection.

\* In this column all positive reactions are indicated by +

test. The volume of the inoculum was 0.1 cc. The dilution used and the results obtained are shown in table 1.

It will be seen from table 1 that all filtrates contained a toxic substance which induced a local erythema. The strength of the filtrates

varied considerably. Only two gave ++ reaction with the 1:1000 dilution, six more gave a ++ reaction at a dilution of 1:500, the remaining thirteen gave ++ reactions only with the 1:100 dilution. One-tenth cubic centimeter of the highest dilution giving a ++ reaction was employed as a skin test dose in subsequent experiments.

*Experiment 2* One skin test dose of each filtrate was injected intracutaneously in Dick positive and Dick negative subjects. All the filtrates gave positive reactions in the Dick positive subjects, negative reactions in the Dick negative subjects (table 1).

From the result of the foregoing experiment it appears that the soluble toxic substance present in all the filtrates, though it causes a local erythema in the skin of individuals who give a positive Dick test, nevertheless fails to do so in persons who give a negative Dick test, when one skin test dose of the filtrate as defined above is employed. While this might seem to indicate that the strains of streptococci from which the filtrates were prepared were *Streptococcus scarlatinae*, neutralization tests described below will show that such a conclusion is not warranted.

*Experiment 3* Each of the twenty-one filtrates was subjected to a neutralization test with known blanching and non-blanching human serums according to the method described above. The results are shown in table 2, the filtrates being grouped according to the results of the neutralization tests.

It will be seen from table 2 that the toxic action of filtrates 1 to 10 was completely neutralized by the blanching serums with a few exceptions in the case of serums IV and V, but that it was not neutralized in any instance by the non-blanching serums. In contrast with this there was no neutralization of filtrates 11 to 21 by either the blanching or non-blanching serums. The failure of complete neutralization by serums IV and V was suspected to be due to a low antitoxin content of these serums. They were consequently tested for their capacity to neutralize standard scarlet fever toxin. Fifteen-tenths of a cubic centimeter failed to neutralize completely ten skin test doses, indicating that the foregoing supposition was correct.

It may be concluded from this experiment that 10 of the 21 strains of *Streptococcus hemolyticus* studied produced toxic filtrates capable of being specifically neutralized by scarlet fever antitoxin. These

ten strains are, therefore, considered to be strains of *Streptococcus scarlatinae*.

As noted above it was found that the filtrates from all strains of *Streptococcus hemolyticus*, whether *Streptococcus scarlatinae* or not,

TABLE 2

*Neutralisation tests with blanching serum containing scarlet fever antitoxin and non-blanching serum containing no antitoxin*

Filtrates	Skin reactions										Fil trates plus 0.85 per cent saline	
	Filtrates plus blanching serums					Filtrates plus non-blanching serums						
	I	II	III	IV	V	I	II	III	IV	V		
1	-	-	-	-	-	+	+	+	+	+	+	
2	-	-	-	-	$\pm$	+	+	+	+	+	+	
3	-	-	-	-	$\pm$	+	+	+	+	+	+	
4	-	-	-	-	0	+	+	+	+	+	+	
5	-	-	-	-	$\pm$	+	+	+	+	+	+	
6	-	-	-	-	-	+	+	+	+	+	+	
7	-	-	-	+	0	+	+	+	+	+	+	
8	-	-	-	+	0	+	+	+	+	+	+	
9	-	-	-	+	0	+	+	+	+	+	+	
10	-	-	-	-	-	+	+	+	+	+	+	
11	+	+	+	+	+	+	+	+	+	+	+	
12	+	+	+	+	+	+	+	+	+	+	+	
13	+	+	+	+	+	+	+	+	+	+	+	
14	+	+	+	+	+	+	+	+	+	+	+	
15	+	+	+	+	+	+	+	+	+	+	+	
16	+	+	+	+	+	+	+	+	+	+	+	
17	+	+	+	+	+	+	+	+	+	+	+	
18	+	+	+	+	+	+	+	+	+	+	+	
19	+	+	+	+	+	+	+	+	+	+	+	
20	+	+	+	+	0	+	+	+	+	0	+	
21	+	+	+	+	0	+	+	+	+	0	+	

- = no reaction complete neutralization.  $\pm$  = slight reaction partial neutralization  
 + = positive reaction, no neutralization 0 = test not done.

gave a positive reaction when one skin test dose was inoculated intracutaneously in individuals with a positive Dick test but no reaction in individuals with a negative Dick test. In view of this result it would seem probable that persons possessing an immunity to

scarlatinal toxin have some immunity to toxins derived from non-scarlatinal strains of *Streptococcus hemolyticus*, or at least have a lower degree of skin reactivity to these toxins than have persons who are susceptible to scarlatinal toxin. In order to test out this assumption the following experiment was done.

*Experiment 4* Eight non-scarlatinal filtrates from cases 11 to 18 were injected in amounts of one, two and five skin test doses in test subject M, who gave a negative reaction to five skin test doses of

TABLE 3  
Skin reactivity of Dick negative persons to culture filtrates of non scarlatinal hemolytic streptococci

Filtrate number	Toxin tests				Neutralization test	
	Skin reactions				Skin reactions in Dick-positive subject	
	In Dick-positive subject 1 STD*	In Dick negative subject M			Filtrate and blanching serum from subject M	Filtrate and non blanching serum
	1 STD	2 STD	5 STD			
11	+	-	±	+	+	+
12	+	-	-	-	+	+
13	+	-	+	+	+	+
14	+	-	-	+	+	+
15	+	-	-	+	±	+
16	+	-	-	+	+	+
17	+	-	+	+	+	+
18	+	-	-	+	±	+
Control with scarlet fever toxin	+	-	-	-	-	+

\* Skin test dose + = positive reaction 1 cm or more in diameter ± = faint reaction less than 1 cm in diameter - = no reaction

standard scarlet fever toxin. The blanching serum from this same subject was used for neutralization tests with these eight filtrates, together with a control non-blanching serum. The results are presented in table 3. This experiment was repeated with some of the filtrates in two other Dick negative subjects with the same result.

From the table it will be seen that 3 of the 8 non-scarlatinal filtrates gave positive reactions in test subject M when two skin test doses were used, 7 of the 8 when five skin test doses were used. It

is furthermore clear from the neutralization tests that the serum from test subject M, though containing a considerable amount of scarlatinal antitoxin, failed to neutralize the toxic action of the non scarlatinal filtrates. From this result it may be concluded that at least some individuals who are immune to scarlatinal toxin as determined by the Dick test, exhibit less skin reactivity to the toxic filtrates from non-scarlatinal hemolytic streptococci than do individuals who are susceptible to scarlatinal toxin. The result, furthermore, emphasizes the necessity for a neutralization test before it can be concluded that a toxin producing strain of hemolytic streptococcus is *Streptococcus scarlatinae*.

#### DISCUSSION

In table 4 are summarized the results of the foregoing experiments together with the data concerning the patients from whom the twenty-one strains of hemolytic streptococci were obtained. The cases are arranged in two groups, group 1 (cases 1 to 10) consisting of those patients in whom it had been found that the infection was due to *Streptococcus scarlatinae*, group 2 (cases 11 to 21) consisting of those patients in whom the infection was due to some other variety of hemolytic streptococcus.

That ten of twenty-one unselected cases of acute streptococcus infection should prove to be infected with *Streptococcus scarlatinae* without any one of these patients developing clinical scarlet fever might seem surprising. It is believed, however, that the explanation for this is found in the data concerning contact with scarlet fever and susceptibility to scarlatinal toxin as determined by the Dick test. It will be seen by reference to Table 4 that nine of these ten patients had a history of direct and fairly intimate contact with scarlet fever. Cases 3 and 10 had children with scarlet fever. Case 5 was nursing two children with scarlet fever. Case 7 had slept with a relative who developed scarlet fever. Case 6 was an interne who developed a severe sore throat one week after serving on a scarlet fever ward. The remaining cases were nurses who had been caring for patients with scarlet fever. In striking contrast with this is the fact that only one of the patients in group 2 had had any known contact with scarlet fever. The source of infection in the patients of group 1, then,

would appear to be satisfactorily explained by their direct exposure to scarlet fever.

In explanation of the fact that none of these ten patients developed clinical scarlet fever, it is to be noted that nine of them, in whom the

TABLE 4

*Occurrence of scarlatinal and non-scarlatinal streptococcus infections in persons without clinical scarlet fever*

Case	Data on patients			Data on streptococci isolated from patients			
	Clinical diagnosis	Contact with scarlet fever	Dick test	Test for toxin production		Neutralization of toxin by	
				Dick positive subject	Dick-negative subject	Blanching serum	Non blanching serum
1	Peritonsillar abscess	+	-	+	-	-*	+-*
2	Tonsillitis	+	-	+	-	-	+
3	Pharyngitis	+	0	+	-	-	+
4	Pharyngitis	?	-	+	-	-	+
5	Tonsillitis	+	-	+	-	-	+
6	Tonsillitis	+	-	+	-	-	+
7	Pharyngitis	+	-	+	-	-	+
8	Tonsillitis	+	-	+	-	-	+
9	Tonsillitis	+	-	+	-	-	+
10	Bronchopneumonia	+	-	+	-	-	+
11	Tonsillitis	-	-	+	-	+	+
12	Septicemia	+	0	+	-	+	+
13	Pharyngitis	-	-	+	-	+	+
14	Sinusitis	-	-	+	-	+	+
15	Tonsillitis	-	-	+	-	+	+
16	Tonsillitis	-	0	+	-	+	+
17	Pharyngitis	-	-	+	-	+	+
18	Pharyngitis	-	-	+	-	+	+
19	Pleurisy	-	-	+	-	+	+
20	Otitis media	-	-	+	-	+	+
21	Pharyngitis	-	0	+	-	+	+

\* - = no reaction, complete neutralization    + = positive reaction, no neutralization    0 = test not done

Dick test was done either before or shortly after the onset of their infection, showed a negative test indicative of an existing immunity to scarlet fever toxin. It seems reasonable to suppose, therefore, that the failure of these patients to develop the clinical picture of

scarlet fever was due to this existing antitoxic immunity. The toxin elaborated at the site of the local tissue infection in the throat or elsewhere would presumably be neutralized locally by the patient's antitoxin. Under these circumstances the specific toxic phase of scarlet fever, which is clinically represented by the early toxemia and the exanthem, would not occur.

Of great interest in relation to the problems of immunity to infection in general and to scarlet fever in particular is the apparent fact that an existing immunity to the soluble toxin of *Streptococcus scarlatinae* does not necessarily prevent the development of even severe local pyogenic infections with this organism in persons in intimate contact with scarlet fever. The epidemiologic and public health problems arising from this fact are obvious and need not be discussed in detail. In brief, it would appear highly probable that the frequency of pyogenic infections by *Streptococcus scarlatinae* is greater than generally supposed, that persons so infected may serve as foci for the spread of scarlet fever, and that a negative Dick test is little or no indication that a person exposed to scarlet fever is not liable to serious pyogenic infections with *Streptococcus scarlatinae*.

#### SUMMARY

Of 21 strains of *Streptococcus hemolyticus* isolated from 21 unselected patients with acute streptococcus infections, 10 were found to be *Streptococcus scarlatinae*. Of the 10 patients in whom the infection was due to *Streptococcus scarlatinae* 5 had acute follicular tonsillitis, 3 had acute pharyngitis, 1 had peritonsillar abscess, and 1 had bronchopneumonia. None developed clinical scarlet fever. Nine of these patients had been intimately exposed to scarlet fever. No information on this point was obtained in the tenth. Nine of them gave a negative Dick test either before or shortly after the onset of the infection. No test was made in the tenth. Of the 11 patients with acute hemolytic streptococcus infections due to non scarlatinal streptococci only one had knowledge of exposure to scarlet fever. None developed scarlet fever. Eight in whom the test was done gave a negative Dick test.

## CONCLUSIONS

1 *Streptococcus scarlatinae* infections without clinical scarlet fever occur with considerable frequency among persons exposed to scarlet fever

2 The failure of persons infected with *Streptococcus scarlatinae* to develop clinical scarlet fever is probably dependent upon the possession of immunity to scarlet fever toxin prior to the onset of the infection

3 Immunity to the toxin of *Streptococcus scarlatinae* as determined by the Dick test, does not necessarily provide the immunity to local pyogenic infections with *Streptococcus scarlatinae*

## BIBLIOGRAPHY

- Bliss, W P Bull Johns Hopkins Hosp, 1920, xxii, 173 A Biological Study of Hemolytic Streptococcus from Throats of Patients Suffering from Scarlet Fever
- Dick, G T, and Dick, G H Jour Amer Med Assoc, 1925, lxxvii, 802 Method of Recognizing Scarlet Fever Streptococcus by Means of Specific Toxin Production
- Hebra Diseases of the Skin Mayr, V, London 1866
- Leichtenstern, O Deutsche Med Wehnschr, 1882, viii, 173 Über die 1880 und 1881 in Köln herrschende Scharlach Epidemic
- Rosenow, E C Jour Amer Med Assoc, 1926, lxxvi, 9 Precipitin Reaction in the Diagnosis of Scarlet Fever and Allied Hemolytic Streptococcus Infections
- Stevens, F A Proc Soc Exper Biol and Med, 1926 xxiii, 346 The Occurrence of Scarlet Fever without a Rash during Epidemics
- Stevens, F A, and Dochez, A R Jour Amer Med Assoc, 1926, lxxvi, 1110 Occurrence of Throat Infections with *Streptococcus scarlatinae* without a Rash
- Stevens, F A Proc Soc Exper Biol and Med, 1926, xxiii, 348 Unusual Instances of Infection with *Streptococcus scarlatinae*
- Thomas and von Ziemssen Cyclopaedia of the Practise of Medicine New York, 1875, ii, 251
- Waring, A J South Med Jour, 1921, xiv, 265 Scarlet Fever and Streptococcus Sore Throat
- Williams, A W Amer Jour Pub Health, 1925, xv, 129 The Relationship between Different Antibodies

## STUDIES IN SCARLET FEVER

### IV POST SCARLATINAL IMMUNITY IN PATIENTS TREATED WITH ANTITOXIN<sup>1</sup>

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#### INTRODUCTION

The present investigation was undertaken to determine, if possible, whether the treatment of scarlet fever with antitoxin has any effect on the degree of permanent active immunity which ordinarily follows an attack of the disease in persons who have not received antitoxin.

The immunity developed by the vast majority of patients convalescent from scarlet fever presumably comes as a response to the presence in the circulating blood during the first week of the disease of the specific toxin produced by the etiological agent, *Streptococcus scarlatinae*. Inasmuch as scarlatinal antitoxic serum, recently introduced for the treatment of scarlet fever (Dochez, 1924), rapidly neutralizes this circulating toxin (Blake, 1924) and thereby removes the stimulus which induces a permanent active immunity, it has seemed possible that patients promptly cured by antitoxin might become susceptible again, after the relatively transient passive immunity conferred by the administration of antitoxic serum had terminated.

Trask and Blake (1924) have demonstrated that the specific toxin of scarlet fever appears in the circulating blood of patients very soon after the onset of symptoms. The greatest number of patients have demonstrable amounts of toxin in the blood on the second and third days of the disease, the incidence declining from this point to the

<sup>1</sup> This work was done with the aid of the Goodhart scarlet fever fund.

<sup>2</sup> This paper is in part a thesis presented to the Graduate School of Yale University in candidacy for the degree of Master of Science.

eighth day after which relatively few patients show toxin in the blood (Blake and Trask, 1926) During this period, in the natural course of events, the immunity mechanism of the body responds to the toxemia by producing autogenous antitoxin which eventually becomes sufficient to neutralize the toxin formed at the site of infection An excess of antitoxin and permanent immunity results in almost all cases not treated with serum Theoretically, if this toxin were artificially neutralized very early by treatment with the specific antitoxin for scarlet fever, it is conceivable that the natural defensive mechanism of the patient would not be sufficiently stimulated to establish an effective permanent immunity

Immunity to scarlet fever is in all probability a relative matter It may conceivably vary with the following factors and possibly with others (1) fluctuations in the amount of immune bodies present at the time of exposure, (2) the site of the area involved in the infection, (3) the local conditions promoting the spread of the infection and rapid absorption of the toxin, such as a concurrent rhinopharyngitis, and (4) the intensity of the "seeding" with *Streptococcus scarlatinae* Bearing this in mind it is evident that a study of the degree of immunity to scarlet fever developed by individuals treated several months previously with scarlatinal antitoxic serum, as contrasted with the immunity developed by those who did not have the benefit of this serum during the stage of specific toxemia, will give only relative and not absolute results

#### SOURCE OF MATERIAL

The patients used consisted of those who responded to a request to return to the clinic for this study The number is not large and represents only a small proportion of the total number of scarlet fever patients treated in the New Haven Hospital since scarlatinal antitoxin was first employed in January, 1924 Most of the cases were children from 2 to 15 years of age In all, 50 former patients returned, 34 of whom had had serum treatment at least five months previously

#### EXPERIMENTAL

Two methods have been used to measure the degree of antitoxic immunity to scarlet fever possessed by the individuals studied The

first method consisted in determining the skin reactivity of the subjects to intracutaneous injections of standard amounts of scarlet fever toxin, the method commonly known as the Dick test. The second method consisted in determining the amount of scarlet fever antitoxin in the subject's blood by using his serum for blanching tests (Schultz Charlton, 1918) and for toxin neutralization tests. The results obtained by the two methods have then been compared in order to determine how closely they agreed in providing a basis for estimating the degree of immunity possessed by each subject.

### *I Skin tests of immunity*

*Methods* The skin tests of immunity were performed according to the accepted method of intracutaneous injection on the flexor surface of the forearm, the readings being made 24 hours after the injection (Dick and Dick, 1924).

The toxin employed in the tests was prepared in this laboratory from a strain of *Streptococcus scarlatinae* supplied by Dr Dochez. Cultures were made in phosphate broth, pH 7.6, to which 2 per cent of defibrinated rabbit's blood had been added. After incubating for four days at 37°C the broth was filtered through a Berkefeld "V" filter. The toxin was standardized according to the method described by Dick and Dick (1925). When diluted 1:2000 with normal saline, 0.1 cc. of the toxin filtrate gave a skin reaction in a series of susceptible individuals approximately equal to the skin reaction produced by the standard skin test dose of toxin (D II) supplied by the United States Hygienic Laboratory in Washington, D.C. This amount was therefore considered to be one skin test dose, the present standard unit of toxin.

In order to determine the relative degree of immunity possessed and to control false reactions three skin tests were done on all cases (1) 0.1 cc. of a 1:500 dilution of toxin equivalent to 4 skin test doses, (2) 0.1 cc. of a 1:2000 dilution, approximately equal to 1 skin test dose, and (3) 0.1 cc. of a 1:500 dilution, heated 10 hours at 100°C.

The skin reactions were interpreted as follows:

+ = positive definite redness with some induration and perhaps tenderness  
the area of erythema being over 10 mm. in diameter

$\pm$  = slightly positive definite erythema approximately 10 mm in diameter, with little or no induration

$\pm$  = faintly or doubtfully positive faint erythema 10 mm or more in diameter

- = negative no erythema or an erythema less than 10 mm in diameter

*Results* The summarized results of the skin tests are shown in table 1, from which it will be seen that the immunity in the cases treated with antitoxin is conspicuously less than it is in those who received no antitoxin. In the treated group only 54 per cent failed

TABLE 1

*Skin tests in former scarlet fever patients 5 months to 2 years after onset of scarlet fever*

Amount of toxin	Skin reaction	Number of cases		Percentage	
		Treated with antitoxin	Not treated with antitoxin	Treated with antitoxin	Not treated with antitoxin
1 S T D	+	9	1	26	6
	$\pm$	7	0	20	0
	$\pm$	0	0	0	0
	-	18	15	54	94
4 S T D	+	18	2	53	13
	$\pm$	7	0	21	0
	$\pm$	1	1	3	6
	-	8	13	23	81
Heat control, 4 S T D	+	1	0	3	0
	$\pm$	2	0	6	0
	-	31	16	91	100

S T D = skin test dose

to react to one skin test dose, in the untreated group 94 per cent. Of the remaining 46 per cent in the treated group 26 per cent gave strongly positive reactions, while in the untreated group only 6 per cent gave strongly positive reactions.

The advantage of employing an additional test of four skin test doses of toxin is obvious, when one considers that one skin test dose gives information over only a relatively small range of immunity. By the use of this larger dose it is even more clear that the susceptibility of the treated cases is greater and their immunity less than is the case with the untreated patients. With four skin test doses the

percentage of strongly positive reactors increases from 26 per cent to 53 per cent, the percentage of negative reactors diminishes from 54 per cent to 23 per cent. In striking contrast with this, it will be seen that the percentages of positive and negative reactors in the untreated group show an insignificant change.

In Table 1 there are included among the cases treated with scarlatinal antitoxin two patients with septic complications who received the serum after the rash had faded and the stage of specific toxemia had passed. Omitting these two cases, one of whom gave a slight combined reaction and the other a positive skin test, the results are not markedly altered. The correction for these two cases has been

TABLE 2  
*Condensed results of skin tests*

Amount of toxin	Skin reaction	Number of cases		Percentage	
		Treated with antitoxin	Not treated with antitoxin	Treated with antitoxin	Not treated with antitoxin
1 S.T.D	Positive	14	1	44	6
	Negative	18	15	56	94
4 S.T.D	Positive	23	2	72	13
	Negative	9	14	28	87
Heat control	Positive	2	0	6	0
	Negative	30	16	94	100

made in table 2, in which the positive (+ and  $\pm$ ) reactions have been combined and the doubtful ( $\pm$ ) reactions have been classified as negative.

Leaving out of account the two cases mentioned as having been treated late in the disease (eleventh and fourteenth days), the interval elapsing between the onset of symptoms and the time of antitoxin treatment varied from less than 24 hours to 8 days, the average time being less than 72 hours. In 13 of the 34 cases, the specific toxin of scarlet fever was actually shown to be present in the blood stream before antitoxin was administered, in four none was demonstrable, while in the remainder the test was not made. The cases varied greatly in severity and in the presence and nature of septic processes.

at the time of treatment. No correlation between these variables and the degree of subsequent immunity could be established.

The contrast between the two series of cases is striking, not only with respect to the reactions to four skin test doses of toxin, but also with respect to the reactions to the standard Dick test of 1 skin test dose. In fact, the percentage of positive reactors (+ and ±) to one skin test dose of toxin among the cases treated with serum, is slightly higher (46 per cent) than that (42 per cent) for a group of eighty student nurses, the majority of whom had never had scarlet fever, tested recently in this hospital with the same toxin. While these results undoubtedly indicate that scarlet fever patients treated with antitoxin develop on the average a less effective active immunity than untreated patients, it nevertheless seems improbable that any considerable number of those showing a + or ± skin reaction would, under circumstances and conditions of exposure, develop a second attack of scarlet fever.

The difference in the two series of cases is too great, however, to leave reasonable doubt as to the greater degree of immunity to scarlet fever toxin developed by the untreated cases, 87 per cent of whom were negative even to 4 skin test doses, as compared with 28 per cent of those who had been treated with antitoxin.

## *II Serum antitoxin tests of immunity*

In order to test the validity of the results of the Dick tests as expressions of immunity to scarlet fever, the minimal blanching dose (M B D) (Blake and Trask, 1925), or the highest dilution of the patient's serum which gave a positive Schultz-Charlton rash extinction test, was determined for seventeen of the cases in the above series. In addition, neutralization experiments were carried out on a smaller number of serums.

*Methods* Approximately 10 cubic centimeters of blood were drawn at the time of the skin tests and the separated serum placed in a refrigerator in a "No-Air" stoppered bottle. A culture was made for sterility.

1 For the blanching tests, 0.5 cc of the undiluted serum or of a 1:10, 1:100, or 1:1000 dilution in normal saline was injected intradermally into the fresh, uniform scarlatinal rash of a patient with

mild or moderate symptoms Repeated readings were made from 12 to 36 hours after injection, the results in most instances being checked by two and sometimes by three observers Complete blanching of the rash at the site of the injection is represented by a double plus (++) The highest dilution showing any blanching is the minimal blanching dose

Since 0.5 cc was injected into the skin, undiluted serum which gave a positive blanching test contained at least 2 M B D per cubic centimeter Similarly, a positive end point in a dilution of 1:10 indicates 20 M B D per cubic centimeter

2 The method for the neutralization tests was similar to that of Henry and Lewis (1925) except that the ratio of serum to toxin was varied in multiples of ten and the amount of toxin was not constant

Three neutralization tests were performed with each serum as follows (1) 0.5 cc toxin, 1:2000 (5 S T D), + 0.5 cc serum, undiluted, complete neutralization indicates at least 0.1 unit of antitoxin per cubic centimeter of serum (2) 0.5 cc toxin 1:200 (50 S T D) + 0.5 cc serum, undiluted, complete neutralization indicates at least 1 unit of antitoxin per cubic centimeter of serum (3) 0.5 cc toxin, 1:200 (50 S T D) + 0.5 cc of serum, 1:10, complete neutralization indicates at least 10 units<sup>2</sup> of antitoxin per cubic centimeter of serum

Dilutions were made with normal saline The mixtures were incubated for 45 minutes in a water bath at 37°C

One tenth of one cubic centimeter (0.1 cc) of each of the three mixtures of serum and toxin and of a toxin control (1:4000) was injected intradermally on the anterior aspect of the arm in test subjects susceptible to scarlet fever toxin It was necessary to employ as a subject an individual who reacted to one-half of one S T D of toxin or less, in order to bring out the reaction to a very small excess of toxin, since the amount of toxin in the first mixture injected was one half of one S T D (0.05 cc of a 1:2000 dilution of toxin)

Readings were made 24 hours after injection, the size and intensity of any erythema which developed and the presence or absence of local swelling or tenderness being noted The apparent dissociation of the toxin antitoxin combination after 24 hours and the late spread

<sup>2</sup> One unit is that amount of antitoxin which neutralizes 100 S T D of toxin

of the zones of erythema, to which Henry and Lewis (1925) called attention, were observed in the majority of the neutralization tests. Experience may show that it is possible to interpolate other propor-

TABLE 3  
*Serum antitoxin tests*

Number	Name	Serum treatment	Serum antitoxin tests						Skin tests		Heat control	
			Minimal blanching doses per cubic centimeter of serum				Neutralization units of antitoxin per cubic centimeter of serum			STD		
			2	20	200	2000	0.1	1	10	4	1	
1	McAvoy	S	-	-	-	-	0	0	0	++	+	-
2	Dunham	S	+	±	-	-	C	0	0	++++	+++	-
3	Franklin	S	±	-	-	-				+	±	-
4	Canby	S	+	-	-	-				++	-	-
5	Clark	S	±	-	?	-				+	+	-
6	Neuman	S	+	+	±	-	C	0	0	+	-	-
7	Schoenrock	S	+	+	±	-	C	C	C	+	+	-
8	Delieto	S	+	+	±	-	C	C	P	±	-	-
9	Hayden	S	++	++	+	-	C	C	C	++	+	-
10	Wilhams	S	++	++	+	-	C	C	P	++	+	-
11	English	S	++	+	±	?				±	-	-
12	Kaplan	S	+	+	+	?				+	-	-
13	Novak	S	+	+	+	?				-	-	-
14	Tulp	S	+	+	+	?				±	-	-
15	Marsagas	S	+	+	+	±	C	C	P	±	-	-
16	Quinn	S	++	+	±	±	C	C	P	-	-	-
17	Nugent	S	++	+	+	+				++	+	-

S = treated with antitoxin    0 = no neutralization    P = partial neutralization    C = complete neutralization

*Skin reaction to toxin*

+++ = erythema 41 to 50 mm in diameter

++ = erythema 31 to 40 mm in diameter

+= erythema 21 to 30 mm in diameter

± = erythema 11 to 20 mm in diameter

± = erythema 10 mm in diameter

± = faintest erythema 10 to 15 mm or more in diameter

- = no erythema or erythema less than 10 mm in diameter

tions of toxin and serum antitoxin so as to obtain closer end-points of neutralization.

*Results* The results of the tests are shown in table 3, together with the results of the skin test for immunity with 4 and 1 skin test

doses of toxin in the same individuals. Fourteen of the persons studied had been treated with antitoxin, three had not. It will be seen that all but one of the group contained demonstrable antitoxin in their serum as determined by the blanching test. Of these, four contained at least 20 M.B.D. of antitoxin per cubic centimeter of serum, nine contained at least 200 M.B.D. and three contained at least 2000. In the eight cases in which neutralization tests were done, seven showed the presence of antitoxin, at least 0.1 unit per cubic centimeter of serum in two cases, at least one unit in three cases, and at least ten units<sup>3</sup> in two cases. These results support the suggestion previously made that it seems improbable that any considerable number of those who show a positive skin reaction would, under ordinary circumstances and conditions of exposure, develop a second attack of scarlet fever.

### *III Comparison of skin and serum antitoxin tests*

Within the limitations of the experiment, there is apparent a fairly close correlation in most of the cases between the skin reactions to toxin and the antitoxin of the serum, whether the latter be determined by means of the M.B.D. or by the end point of neutralization. The lack of agreement in Number 7 may possibly be explained on the basis of a slightly positive heat control or pseudo-reaction. Whether or not another control skin test would prove Number 10 to be allergic to a non specific substance in the broth filtrate has not been determined. Number 17 is another instance of disagreement between the results of the skin test and the antitoxin content of the serum. A re test with a neutralized control instead of a heat control was refused. With these three exceptions, in no instance did the serum of an individual giving a positive skin reaction to one S.T.D. contain as much as 200 M.B.D. of antitoxin per cubic centimeter, while those giving a negative skin test to one S.T.D. all except Number 4 showed 200 M.B.D. or more of antitoxin per cubic centimeter.

It will be seen that the correlation between the skin reactions and the antitoxin content of the serum, as determined by either method, is not as close as that between the results of the blanching and of the neutralization experiments, which agree fairly well.

## SUMMARY

A comparison has been attempted between the degree of late immunity to scarlet fever developed by (a) former patients who were treated with scarlet fever antitoxin during the toxemic stage of the disease and (b) those who did not receive antitoxin.

Fifty cases are presented, 34 of whom had received antitoxin and 16 no antitoxin. The skin reactivity to toxin, after an interval of from five months to two years after the onset of the disease, was determined in terms of the response to four S T D , one S T D , and a heat control of four S T D . Further experiments correlate the skin tests for immunity with the antitoxin content of the serum, as expressed in minimal blanching doses and in the end-points of neutralization of toxin.

## DISCUSSION

Immunization to scarlet fever, in response to the stimulus of circulating toxin undoubtedly takes place, in most instances, very rapidly. Whether or not the advent of scarlet fever antitoxin therapy will so alter the natural course of immunization to the disease as to increase the incidence of second attacks of scarlet fever in individuals treated with serum, experience will show. Probably only a small percentage of those individuals in the series, in whom the Dick test remains positive, would, under ordinary conditions of exposure, contract scarlet fever. An analogous condition arises from the partial, active immunization of individuals with injections of toxin. In several instances (Dick and Dick, 1925) these individuals have subsequently contracted the disease in mild form.

Perhaps, in some instances, of relapse following inadequate antitoxin therapy is the result of the early temporary neutralization of the toxin and consequent removal of the natural stimulus to antitoxin formation.

## CONCLUSIONS

1 The degree of late immunity to scarlet fever developed by patients treated with adequate therapeutic doses of scarlet fever antitoxin during the toxemic stage of the disease appears to be less than that developed by patients who did not receive antitoxin.

2 A correlation exists between the results of the skin tests of immunity to scarlet fever and the antitoxin content of the serum, whether the amount of serum antitoxin be determined by blanching or by neutralization experiments

#### BIBLIOGRAPHY

- Blake F G Trans Assoc Amer Phys, 1924, xxxix 141 Observations on the Treatment of Scarlet Fever with Scarlatinal Antistreptococcal Serum
- Blake F G, and Trask, J D Bost Med. and Surg Jour, 1925, cxcm, 659 The Treatment of Scarlet Fever with Antitoxin
- Blake, F G , and Trask J D Jour Clin Invest, 1926, in, 397 Studies in Scarlet Fever II The Relation of the Specific Toxemia of Scarlet Fever to the Course of the Disease
- Dick, G F , and Dick, G H. Jour Amer Med Assoc, 1924, lxxxii 265 A Skin Test for Susceptibility to Scarlet Fever
- Dick G F , and Dick G H Jour Amer Med Assoc, 1925, lxxxiv, 1477 Results with the Skin Test for Susceptibility to Scarlet Fever
- Dochez, A R. Trans. Assoc Amer Phys., 1924 xxxix, 136 Studies in Scarlet Fever
- Henry, H., and Lewis, F C Lancet, 1925, ii, 587 On Immunity to the Toxin of *Streptococcus scarlatinae*
- Schultz and Charlton Ztschr F Kinderh 1918, Orig , xvii, 328
- Trask, J D , and Blake F G Jour Exper Med., 1924 xl 381 Observations on the Presence of a Toxic Substance in the Blood and Urine of Patients with Scarlet Fever



# THE EFFECT OF TACHYCARDIA ON THE BLOOD FLOW IN DOGS

## I THE EFFECT OF RAPID IRREGULAR RHYTHMS AS SEEN IN AURICULAR FIBRILLATION

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The frequent occurrence of tachycardia, both regular and irregular, in patients makes it desirable to have exact information as to the effect of these rhythms on the circulation. Up to the present few studies have been directed toward this end either in patients or in experimental animals, because of the difficulty in the first place of obtaining samples of mixed venous blood and in the second place of inducing these rhythms under conditions which were not too far removed from normal conditions.

Stewart (1) in a study of the oxygen saturation of the arterial and of the venous arm blood in patients during auricular fibrillation and after the return to the normal rhythm under quinidine sulphate, found that during the period of fibrillation the arterial oxygen saturation was practically unchanged, while that of the venous blood was decreased. It appeared therefore that the blood flow was slower during auricular fibrillation than during the normal rhythm. Goldschmidt and Light (2) have shown that the oxygen saturation of the venous arm blood varies with temperature and to some extent with position, but the consistency in the changes which Stewart observed when these factors were taken into account make it seem unlikely that these two factors were responsible for them. Meakins (3) found that neither regular nor irregular tachycardia affected the oxygen saturation of the arterial blood in dogs. There is a difficulty however in the interpretation of Meakins' experiments, for the dogs were under paraldehyde anesthesia, the chests were open and the dogs were kept alive by means of artificial respiration. In cases

where the rhythm is regular Carter and Stewart (4) have likewise reported marked anoxemia in a patient during paroxysms of auricular tachycardia and Dieuaide (5) has reported a similar state in a patient during attacks of paroxysmal ventricular tachycardia. On the other hand in Barcroft, Bock, and Roughton's (6) patient the oxygen saturation of the arterial blood was normal during paroxysmal auricular tachycardia. These variations in the observations so far reported leave the subject unsettled. In the work which we are now reporting we have studied the effect of induced tachycardia under physiological conditions and have been able to make the observations on the mixed venous blood. In this paper we shall report experiments on the effect of auricular fibrillation, as a type of irregular tachycardia, on the blood flow in normal dogs, while in a second paper we shall report other experiments on the effect of regular tachycardia.

#### METHODS

Dogs of diverse weights were used in these experiments. The animals were prepared 24 hours before the experiments by the operation which is now described. The operations were carried out under sterile precautions. Ether was given by the intratracheal method. With the dog lying well on his left side the skin and muscles were incised to expose the fourth right rib. About 6 cm of this rib was removed and the heart exposed through this opening. In the later experiments ample exposure of the heart was obtained simply by making an incision through the interspace between the fourth and the fifth ribs. A small sand bag placed under the dog's chest helped to give a good exposure. The lobes of the right lung were packed aside with squares of black Japanese silk moistened with warm physiological salt solution. An incision 3 cm in length was made over the right auricle parallel to the auriculo-ventricular groove and about 0.5 cm above it. A special wire electrode was sutured to the auricular wall about 0.5 cm above the auriculo-ventricular groove (fig 1). A second electrode was sutured 1.0 cm above the first. The wire electrodes were made of No. 30 gauge brass wire about 20.0 cm long. At one end the wire was coiled into a loose spiral of 8 to 10 turns. The spiral portion was 3.0 mm in diameter and 1.5 cm in length. The spiral ends were sutured to the auricle. The placing of the two spirals with reference to each other is illustrated in fig 1. By this arrangement a short circuit is less likely to occur during stimulation through these electrodes. Three or four sutures of fine black silk doubled were sufficient to fix each electrode to the muscle. Fine curved needles were used. The four sutures were first placed in the auricular muscle, then the spiral electrode was held in place and the sutures tied about the spirals. Hemorrhage did not occur in placing the sutures in the auricular muscle. The wires

leading from the spirals were insulated with small fine bore rubber tubing down to their attachment to the muscle. The pericardium was closed, the insulated wire brought outside the chest wall, and the chest closed tightly in layers after dis-tension of the lungs to expel all free air from the pleural cavity. The whole operation required about three quarters of an hour from the time the administration of ether was begun until it was discontinued. The depth of anesthesia was kept as light as possible. The dogs were given morphine 32.0 mgm hypodermically before being removed from the operating table. The dogs recovered from the ether within one to two hours. The operations were performed in the morning or early afternoon and by evening the dogs were walking about the cage. Care was exercised throughout the operation to prevent injury to the tissues.

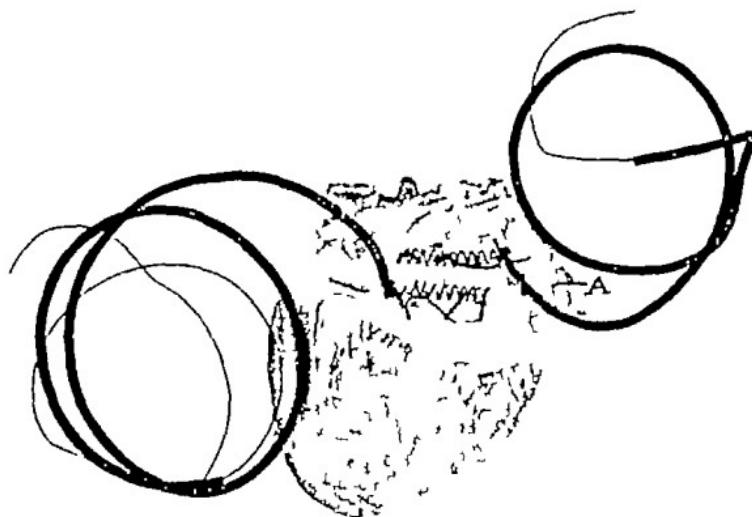


FIG 1 This photograph shows the position in which the spiral wire electrodes are sutured to the right auricle. A is the right auricular appendage.

#### PLAN OF OBSERVATIONS

The day after the preliminary operation the following experiment was performed. The dogs lay quietly on the animal table during these experiments *without anesthesia*. Non polarizable electrodes were placed on the right fore leg and the left hind leg for the derivation of Lead II of the electrocardiogram. Samples of arterial and of mixed venous blood were drawn during the presence of the normal rhythm. The arterial blood was drawn from a femoral artery. The

samples of mixed venous blood were obtained by inserting a special cannula into the right ventricle through the right external jugular vein (Stewart (7)) Novocaine 1 to 2 per cent was injected into the skin of the neck before exposing the vein. The right auricle was then stimulated through the wire electrodes by faradic current, and made to fibrillate for 30 to 90 minutes. The faradic current was obtained from one or two dry cell batteries in the primary circuit of a Du Bois-Reymond induction coil. At the end of the fibrillatory period and while the auricles were still fibrillating second samples of arterial and of mixed venous blood were drawn. Third samples were taken several hours after the end of the stimulation period. The oxygen contents of the blood samples were estimated in duplicate by the Van Slyke and Neill manometric method (8) immediately after the samples were taken. The oxygen capacities of the arterial and in some instances of the mixed venous blood were ascertained for the calculation of the degree of oxygen saturation. Electrocardiograms were made at the time the blood samples were taken in order to be certain of the cardiac rhythm then prevailing and to estimate the heart rate.

The oxygen consumed by the tissues per liter of blood was obtained by subtraction of the oxygen content of the mixed venous blood from the oxygen content of the arterial blood. By calculating the ratio of the oxygen consumed per liter of blood during the fibrillatory period to the oxygen consumed during the normal rhythm we obtained the relative blood flow during the induced rhythm, the ratio during the normal control period being placed at 100. Since the dog lay quietly on the board during the experiment and had not been given food on the day of the experiment we have assumed that the changes in oxygen consumed per liter of blood were due to changes in blood flow, and not to changes in metabolism. We did not attempt to estimate the oxygen absorption of the dogs because of the wide variation in the results obtained in untrained, unanesthetized dogs. We therefore were unable to calculate in absolute figures the minute volume output by the heart or the output per beat.

#### OBSERVATIONS

We have studied the effect of auricular fibrillation on the blood flow in 10 dogs. Two additional experiments serve as controls.

*The effect of auricular fibrillation on the oxygen saturation of the arterial blood* The oxygen saturation of the arterial blood was unchanged in 4 observations, increased 4 per cent in one observation and decreased 2 to 3 per cent in 5 observations (table 1) None of these changes were of greater magnitude than the changes found in the two controls, namely, a 6 per cent increase and a 1 per cent decrease. On the whole therefore auricular fibrillation has no effect on the oxygen saturation of the arterial blood

*The effect of auricular fibrillation on the oxygen saturation of the mixed venous blood* The oxygen saturation of the mixed venous blood was decreased in every observation (table 1) The decrease varied between 12 and 35 per cent in 8 observations and was 7 and 9 per cent in the other two observations With the return to the normal rhythm the saturation again increased In dog 167 auricular fibrillation persisted, and in this instance the oxygen saturation of the mixed venous blood remained low In the two controls there was an increase of 5 per cent in one and a decrease of 3 per cent in the other

*The effect of auricular fibrillation on the blood flow* The blood flow in dog 157 was decreased 36 per cent during the period of auricular fibrillation, and began to return toward normal as soon as fibrillation stopped (table 1 and fig 2) The rhythms which obtained at the time that the blood samples were taken in this dog were recorded electrocardiographically (fig 3) In this case the return to normal rhythm did not take place at once, but passed through that of auricular flutter, a mechanism closely allied to fibrillation (fig 3c) In dog 167 (table 1), spontaneous auricular fibrillation was still present two and one half hours after discontinuance of the faradic stimulation, in this case the blood flow showed a further decrease Similar decreases in blood flow occurred during auricular fibrillation in all of the 10 observations (table 1) The blood flow during the fibrillatory period was only 80 to 38 per cent of what it was during the normal rhythm, that is to say, the blood flow was decreased between 20 and 62 per cent In 7 observations (dogs 157, 167, 169, 194, 197, 198, and 151) the blood flow was decreased more than 30 per cent, in the other 3 animals (dogs 193, 195, 199) the decrease was between 20 and 30 per cent

*The effect of auricular fibrillation on the blood flow in dogs*

195	12.4	Before	10.92	6.89	4.03	100	20	11.94	90.2	57.0	N.R.	180
		During	10.85	5.85	5.00	80			90.1	48.6	A.F.	300-320
		After	10.60	7.01	3.59	112	11.21	93.8	62.2	N.R.	60	180
197	25.8	During	9.54	5.16	4.38	66	34	10.41	90.8	49.2	A.F.	60+
		After	9.12	6.21	2.91	100	9.56	94.4	64.5	N.R.	280	186
198	16.0	Before	9.19	7.15	2.04	100		9.77	93.2	72.8	N.R.	140
		During	9.72	5.05	4.67	44	56	10.35	93.0	48.4	A.F.	60
		After	9.47	6.57	2.90	70		9.78	95.7	66.7	N.R.	280-300-320
199	18.7	Before	9.86	7.50	2.36	100		10.52	92.8	70.9	N.R.	140
		During	10.00	6.92	3.08	77	23	10.71	92.5	64.2	A.F.	60
		After	10.24	7.31	2.93	80	10.71	94.8	67.9	N.R.	330	236
154	14.6	Before	7.77	4.98	2.79	100		8.49	90.5	53.2	N.R.	40
(Con-		During	7.86	5.18	2.68	104		8.02	96.8	63.6	N.R.	300
trol)											Reg.	300
189	8.8	Before	6.48	3.75	2.73	100		7.12	89.8	52.1	N.R.	210
(Con-		During	6.43	3.57	2.86	95	7.16	88.6	49.3	Reg.	340	100
trol)											162	

\* N.R. = normal rhythm.

† A.F. = auricular fibrillation.

‡ 2 1 F1 = 2 1 auricular flutter.

§ This femoral rate was counted from a smoked drum record.

¶ Spontaneous auricular fibrillation was present in this dog when blood samples were taken. We did not know how long it had been present. We therefore allowed it to continue for 1 hour longer under faradic stimuli. The control blood samples were taken 4 hours after the end of the fibrillary period.

To convert  $\text{mM O}_2$  to  $\text{millies per cent}$  multiply the values by 2.24.

Before calculating the oxygen saturations, 0.09  $\text{mM}$  and 0.04  $\text{mM O}_2$  (the amounts of oxygen in physical solution) were subtracted from the arterial and mixed venous oxygen contents respectively.

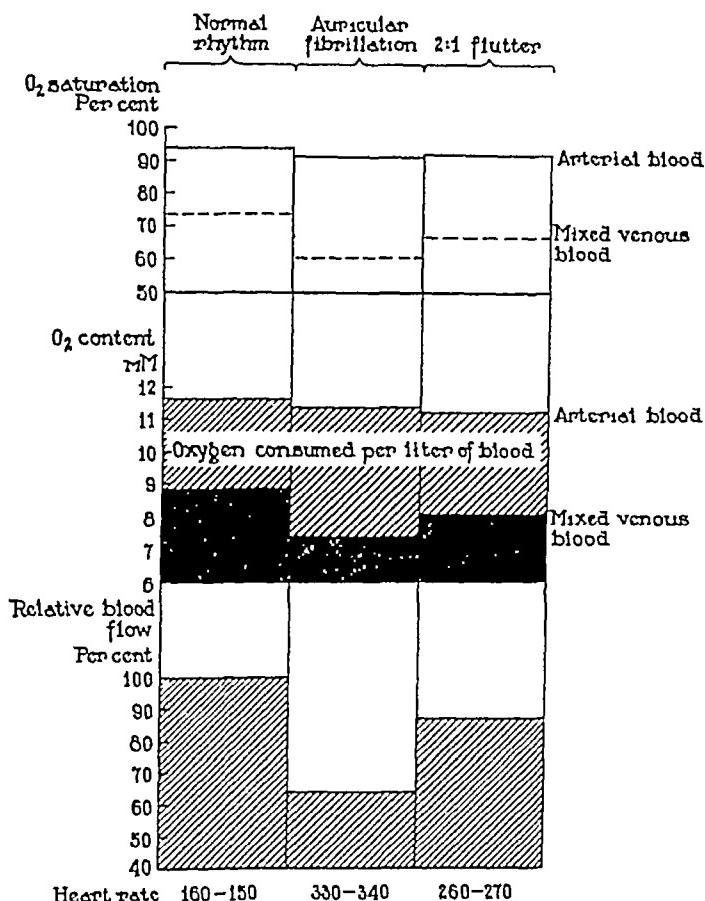


FIG 2 The effect of irregular tachycardia (auricular fibrillation) on oxygen saturation of the arterial and of the mixed venous blood and on the blood flow in dog 157 is indicated graphically in this figure. The oxygen saturation of the arterial blood was unchanged during the fibrillatory period, while that of the mixed venous blood was decreased. The oxygen consumed by the tissues per liter of blood was increased, the blood flow was decreased 36 per cent. When fibrillation stopped the blood flow increased, the oxygen consumed per liter of blood decreased, and the oxygen saturation of the mixed venous blood increased. The return to normal rhythm did not take place at once, but passed through that of auricular flutter (fig 3). The ventricular rate prevailing at the time the blood samples were taken is shown at the bottom of the figure.

Dog 154 (table 1) serves as a control. After stimulation for 1 hour the blood flow had not changed and on analysis of the electrocardio graphic records it was found that the rhythm had not been influenced.

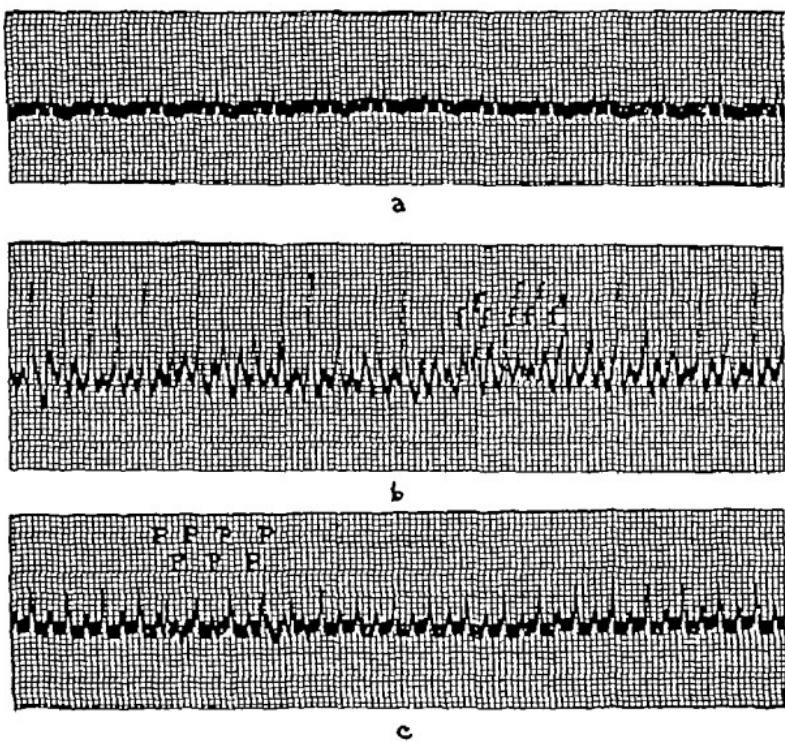


FIG 3 Electrocardiograms of dog 157 are shown at the time the blood samples were drawn. 3a was made during the normal rhythm. 3b during auricular fibrillation (fff are fibrillation waves). 3c was taken 2 hours after the end of stimulation and shows that 2 1 auricular flutter was present (PPP are flutter waves). Lead II of the electrocardiogram is reproduced. Divisions of the ordinates equal  $10^{-4}$  volts. Divisions of the abscissae equal 0.04 of a second. The original curves are sharply contrasted black and white no half tones are lost by the method of reproduction here used. The electrocardiograms are reduced to two-thirds of their natural size.

by the stimulation. At autopsy it was found that one of the wire electrodes was broken inside the rubber insulating tube so that the circuit was broken and current did not reach the heart. Dog 189 (table 1) is the other control. During faradic stimulation a regular

tachycardia developed instead of auricular fibrillation. In this dog there was no change in blood flow.

In these experiments then the blood flow was decreased in 10 dogs during auricular fibrillation while the two controls failed to show such a change.

#### DISCUSSION

The results of these experiments indicate that the blood flow is decreased during auricular fibrillation. This conclusion was arrived at from a comparison of the oxygen consumed by the tissues per liter of blood during the normal rhythm and during auricular fibrillation. The dogs lay quietly on the board during the experiment without anesthesia or sedatives. They were given no food on the day of the experiment. Under these conditions the tissue requirements in all probability remain unchanged. On the basis of these considerations we believe that we are justified in interpreting the changes in the oxygen consumed as being due to changes in blood flow. We think of the events as follows. If the oxygen requirements of the tissues remain unchanged and the same amount of blood is brought to a given part in a unit of time, the same amount of oxygen should be removed from the blood, while if the blood flow is slower more oxygen should be removed. It would have been desirable to ascertain the minute volume output of the heart or the output of the heart per beat rather than the change in blood flow, but for making this calculation we have not the requisite data. To obtain this information it would have been necessary to measure the oxygen absorbed per minute by the dogs during each variety of cardiac rhythm. But to do so masks must be put on the dogs. When they are untrained and unanesthetized there is in consequence a disturbance which brings about wide variation in the results. On the other hand we did not wish to anesthetize the animals because of the secondary changes that accompany anesthesia. Because of the undesirable effect of morphine on the respiration we decided against the use of this drug.

The results of these experiments are in harmony with the results which Blumgart and Weiss (9) have obtained in patients by another method. They measured the time required for radium "c" to pass from the vein of one arm to the artery of the other arm (circulation time) and found that this is much increased in auricular fibrillation.

We are next concerned with analyzing the reason for the decreased blood flow during auricular fibrillation. Among the factors that may be operative is the ineffectiveness of many of the ventricular beats which fail of expelling blood into the arteries, that is to say a pulse deficit may be present. We know that a pulse deficit is present during auricular fibrillation in many patients, it seems likely that it may also occur during experimental conditions. Experiments are now in progress to ascertain this point.

In spite of the fact that during auricular fibrillation the mixed venous blood reaching the lungs is much more unsaturated than during the normal rhythm, the oxygen saturation of the arterial blood is unchanged. This state of affairs is due probably to the combination of two factors. In the first place under normal conditions the mixed venous blood is quickly saturated with oxygen in the lungs with time to spare to take care of blood that is even more unsaturated. In the second place, since in auricular fibrillation the blood flow is decreased 20 to 62 per cent, the blood remains in the lungs a correspondingly longer time and in this way the more unsaturated mixed venous blood is raised to the same level of saturation as obtains during the normal rhythm. Stewart (10) has found that there is no change in the oxygen saturation of the arterial blood in patients with auricular fibrillation when the ventricular rate is increased following the injection of atropine.

In chronic cardiac disease the hemoglobin content of the blood is often increased. A compensatory mechanism is in this manner brought about which facilitates the transport of oxygen. In patients a long time is required for its development. In these experiments there was no consistent change to correspond with this in the hemoglobin content (oxygen capacity) of the blood during auricular fibrillation.

#### SUMMARY

The blood flow and oxygen saturations of the arterial and of the mixed venous blood has been studied during experimental auricular fibrillation in 10 normal unanesthetized dogs. It was found that

1. The blood flow decreased 20 to 62 per cent during auricular fibrillation.

2 The oxygen saturation of the arterial blood was unchanged during auricular fibrillation

3 The oxygen saturation of the mixed venous blood decreased during auricular fibrillation

4 Following the return of the heart to the normal rhythm the oxygen saturation of the mixed venous blood and the blood flow return toward normal. In one instance in which spontaneous auricular fibrillation persisted there was no such tendency

5 The changes described as occurring during auricular fibrillation did not occur in two control experiments

#### CONCLUSIONS

1 The heart during auricular fibrillation is less effective in the propulsion of blood than it is during the normal slow rhythm

2 Auricular fibrillation *per se* does not affect the oxygen saturation of the arterial blood

#### BIBLIOGRAPHY

- 1 Stewart, H J Arch Int Med, 1923, **xxi**, 871 Observations on the Blood Gases in Auricular Fibrillation and after the Restoration of the Normal Mechanism
- 2 Goldschmidt, S, and Light, A B Amer Jour Physiol 1925, **lxxiii**, 146 The Effect of Local Temperature upon the Peripheral Circulation and Metabolism of Tissues as Revealed by the Gaseous Content of the Venous Blood  
Goldschmidt, S, and Light, A B Amer Jour Physiol, 1925, **lxxiii**, 127 A Comparison of the Gaseous Content of Blood from Veins of the Forearm and the Dorsal Surface of the Hand as Indicative of Blood Flow and Metabolic Differences in These Parts
- 3 Meakins, J Heart, 1921-22, **ix**, 185 The Influence of Circulatory Disturbances on the Gaseous Exchange of the Blood I The Oxygen Saturation of the Arterial Blood in Tachycardia
- 4 Carter, E P, and Stewart, H J Arch Int Med, 1923, **xxi**, 390 Studies of the Blood Gases in a Case of Paroxysmal Tachycardia
- 5 Dieuaide, F R Johns Hop Hosp Bull, 1924, **xxv**, 229 Observations on the Respiratory Gases in Ventricular Paroxysmal Tachycardia
- 6 Barcroft, J, Bock, A V, and Roughton, F J Heart, 1921-22, **ix**, 7 Observations on the Circulation and Respiration in a Case of Paroxysmal Tachycardia

- 7 Stewart, H J *Jour Biol Chem*, 1925, lxii, 641 The Oxygen and Carbon Dioxide Contents of the Arterial and Mixed Venous Blood in Normal Intact Dogs.
- 8 Van Slyke D D, and Neill, J M *Jour Biol. Chem*, 1924, lxi, 523 The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement. I.
- 9 Blumgart, H, and Weiss S *Jour Clin Invest*, 1926, ii, 600 The Velocity of Blood Flow in Health and Disease
- 10 Stewart, H J *Jour Clin Invest*, 1926, m, 241 The Effect of Increased Heart Rate Due to the Injection of Atropine on the Oxygen Saturation of the Arterial and Venous Blood of Patients with Heart Disease



# THE EFFECT OF TACHYCARDIA ON THE BLOOD FLOW IN DOGS

## II THE EFFECT OF RAPID REGULAR RHYTHM

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In the first paper of this series (1) were reported observations on the effect of *irregular* tachycardia (auricular fibrillation) on the blood flow in dogs. It was found that the blood flow was decreased during this mechanism. Observations on the effect of *regular* tachycardia form the subject of this paper.

The operative procedure used in the preparation of the dogs and the method of investigation were described in the preceding paper. Briefly, wire electrodes were sutured to the right auricles. The operations were performed with sterile precautions. The dogs were anesthetized with ether given by the intratracheal method. After the dogs recovered from the preliminary operation the heart was stimulated through these electrodes and the effect of the induced rhythm on the blood flow was studied. The regular tachycardias were induced by means of single induced break shocks thrown into the auricle at a regular rapid rate, which could be varied as desired. We have used rates between 250 and 400 per minute. The apparatus (fig 1) which we used to obtain these stimuli was essentially the same as that used by Cohn and Levy (2) in studying the effect of quinidine sulphate on the refractory period of the heart muscle in dogs. Only break shocks were used, the make shocks being short-circuited. The induced current was obtained from two dry cell batteries inserted in the primary circuit of a Du Bois Reymond induction coil. The rate of the induced shocks was recorded by the shadow of the string of a second galvanometer on the same photographic film on which the electrocardiogram was photographed. An electromagnetic signal was placed in the primary circuit and indicated on the film when the

stimuli began to be thrown in and when they were discontinued The rhythm was followed constantly by watching the shadow of the

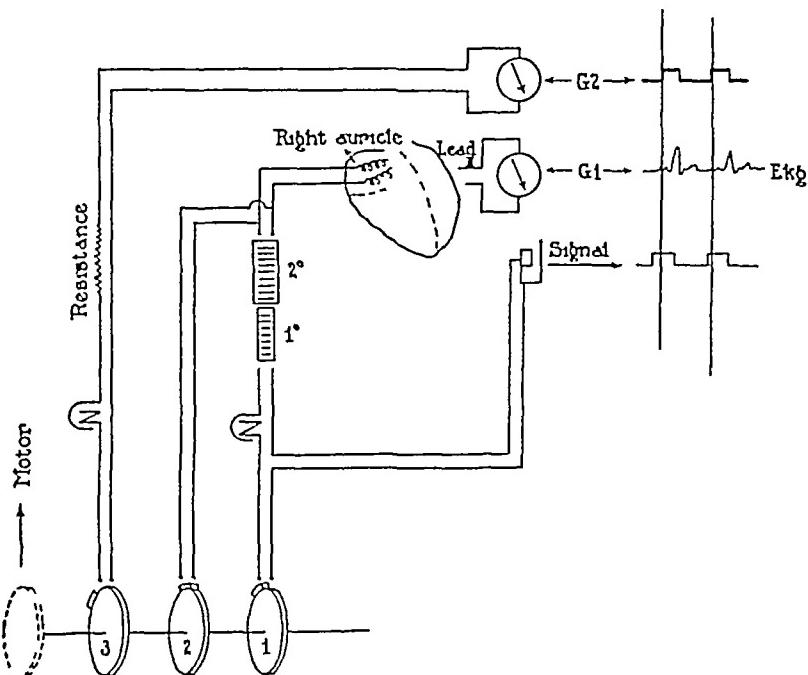


FIG 1 A diagram is shown of the apparatus used to obtain regular rapid induced "break" stimuli. 1, 2 and 3 are wheels arranged on a shaft which is driven by a motor, the speed of which can be varied.  $1^\circ$  and  $2^\circ$  are the primary and secondary coils respectively of the Du Bois-Raymond induction apparatus.  $G_1$  and  $G_2$  represent string galvanometers. The cog on wheel 2 is placed slightly ahead of the cog on wheel 1. By this arrangement, when the wheels revolve the induced "make" shock resulting from the contact made by cog 1 is short-circuited by cog 2. By the time the cogs have moved around so that the "break" shock occurs the cog on wheel 2 has also moved on and leaves the secondary circuit open, the induced "break" shock passing on to the heart. The cog on wheel 3 is placed so that it makes a contact affecting  $G_2$  at the instant the "break" shock occurs. The electromagnetic signal is inserted in the primary circuit. In the apparatus we have used there are three cogs on each wheel, the speed of revolution of the shaft was regulated by gearing pulleys of various size, the speed of the motor could be varied by a resistance coil.

galvanometer string and records were made frequently. When regular tachycardias and auricular fibrillation were induced in the same animal, faradic current as before was used to induce the irregular

rhythm (auricular fibrillation) In some instances the regular tachycardia was induced first, while in other experiments the observations were made first during the period of fibrillation, after a rest the heart was driven at the same ventricular rate but with a regular rhythm Oxygen contents of the arterial and of the mixed venous blood were estimated three times during the normal rhythm, when the induced rhythm had been present for one hour, and a third time several hours after the stimulation had been discontinued As before, the dogs lay quietly on the table without anesthesia and food was not given on the day of the experiment Under these conditions we have interpreted changes in the oxygen consumed per liter of blood (that is to say, the difference between the oxygen content of the arterial and of the mixed venous blood) as representing changes in blood flow The ratio of the oxygen consumed in the two periods gives then the relative blood flow during the two periods This method of interpretation is discussed at greater length in the preceding paper

In a few experiments we have more than one observation on the effect of regular tachycardia on the blood flow In 5 animals we have been able to compare the effect of regular tachycardia with that of irregular tachycardia (auricular fibrillation) at the same absolute ventricular rate per minute or at comparable rates A period of rest followed each period of tachycardia in order to allow the blood flow to return toward normal if there had occurred any change, and also in order that there should be no cumulative effect from prolonged stimulation

#### OBSERVATIONS

We have 16 observations on 9 dogs showing the effect of regular tachycardia on the circulation

*The effect of regular tachycardia on the oxygen saturation of the arterial blood* In 11 observations in 7 dogs the oxygen saturation of the arterial blood was unchanged (tables 1, 2 and 3) while in 5 observations in 4 dogs (approximately one third of the observations) there was an unimportant decrease in saturation ranging from 4 to 7 per cent. There were no increases beyond 3 per cent. There was then no consistent change in the arterial oxygen saturation Usually it was unchanged although occasionally a slight decrease occurred

TABLE I  
*The effect of regular tachycardia on the blood flow in dogs*

Dog-number	Weight kgm.	Time with reference to stimulation	O <sub>2</sub> content		Blood flow per cent of initial	Change in blood flow* per cent	O <sub>2</sub> capacity ml. per liter of blood	O <sub>2</sub> saturation ‡		Rhythm	Duration of stimula- tion minutes	Heart rate per minute	Heart rate per cent of initial	Duration of rest hours
			mL	mL				Arterial	Mixed venous					
189	8.8	Before	6.41	4.32	2.09	100	7.22	87.5	59.3	N R	160-170	175	3	
		During	6.62	4.12	2.50	84	7.20	90.7	56.6	N R	250-280	162		
		Before	6.48	3.75	2.73	100	7.12	89.8	52.1	N R	210	340		
		During	6.43	3.57	2.86	95	7.16	88.6	49.3	Reg tachy†	80	120		
77	7.7	Before	5.91	2.89	3.02	100	6.45	90.2	44.2	N R	210	240-250‡	22	
		During	5.71	2.61	3.10	97	6.33	88.8	40.6	N R	38	120		
190	14.6	Before	10.71	7.67	3.04	100	11.16	95.2	68.4	N R	160	300-320	200	
		During	10.07	6.72	3.35	91	10.99	90.8	60.8	N R	33	180		
144	14.4	Before	10.29	6.34	3.95	100	10.39	98.2	60.8	N R	73	300-310	163	
		During	9.54	5.51	4.03	98	-2	10.39	90.9	52.6	N R	190	180	
		After	8.86	5.06	3.80	104	+4	9.95	88.1	50.4	N R	180	180	
191	14.9	Before	9.72	7.59	2.13	100	10.11	95.3	74.7	N R	70	170-180	188	
		During	9.50	7.00	2.35	91	-9	9.82	95.8	72.4	N R	250	250	
		After	9.39	7.00	2.39	89	-11	9.88	94.1	70.4	N R	50	200-310-320	
		Before	9.39	7.00	2.39	100	-4	9.88	94.1	70.4	N R	250	250	80-128
		During	9.22	6.74	2.48	96	-4	9.72	93.9	68.9	N R	60	160	
		Before	7.55	6.15	1.40	100	-3	7.90	94.4	77.3	N R	310	100	
		During	7.56	6.12	1.44	97	-3	8.13	91.8	74.7	N R	60	193	

196	9 8	Before	9 81	6 87	2 94	100	10 18	95 5	67 1	N R	220	280	173	
		During	9 17	3 93	5 24	56	-44	9 57	40 6	N R	60	380		
		After	8 95	5 70	3 25	90	-10	9 14	61 9	N R	240			13
		Before	8 95	5 70	3 25	100		9 14	66 9	N R	240			
		During	8 68	3 26	5 42	60	-40	8 95	35 9	N R	60	350		150
		After	7 96	2 61	5 35	61	-39	8 56	11 9	Vent.	2	360		
									tachy					14
									N R		240			

\* In this table and in table 2 + in this column indicates increase and - decrease.

† Faradic stimulation

‡ No response to stimuli

§ Paroxysm of ventricular tachycardia at end of record.

¶ Before calculating the oxygen saturations in this table and in table 2, 0.09 mM and 0.04 mM O<sub>2</sub> (the amounts of oxygen in physical solution) were subtracted from the arterial and mixed venous oxygen contents respectively

TABLE 2  
*A comparison of the effect of regular and irregular tachycardia (auricular fibrillation) on the blood flow in dogs*

Dog number	Weight	Time with reference to stimulation	O <sub>2</sub> content		Blood flow per cent of initial	Change in blood flow per cent	O <sub>2</sub> capacity	O <sub>2</sub> saturation	Rhythm	Duration of stimulation minutes	Heart rate per minute	Heart rate per cent of initial	Duration of rest hours	
			Arterial mM	Mixed venous mM										
198	16.0 kgm	Before	9.19	7.15	2.04	100	9.77	93.2	N R	60	280-300-320	214	24	
		During	9.72	5.05	4.67	45	-55	10.35	93.0	A T	60	170		
		After	9.47	6.57	2.90	70	-30	9.78	95.7	N R	60	140		
		Before	9.47	6.57	2.90	100	0	9.78	95.7	N R	60	170		
		During	9.13	6.22	2.91	100	0	10.08	89.7	N R	80	320-330	194	
		After	9.35	5.98	3.37	100	0	9.66	95.8	N R	60	120	229	
193	18.2 kgm	Before	9.08	5.44	3.64	92	-8	9.88	90.9	N R	60	270-280-290	229	2
		During	9.25	5.61	3.64	92	-8	9.82	93.3	N R	60	150	150	
		After	9.00	5.06	3.94	92	-8	9.91	91.9	N R	80	260-370	210	
		Before	9.25	5.61	3.64	100	0	9.82	93.3	N R	80	150	190	
		During	9.25	4.19	5.06	72	-28	9.98	91.8	A F	80	150	190	
		After	9.00	5.06	3.94	92	-8	9.91	91.9	N R	80	260-370	210	
197	25.8 kgm	Before	9.97	7.12	2.85	100	0	10.84	91.1	N R	60	180	180	4
		During	9.75	3.42	6.33	60	-40	10.47	92.3	N R	60	370-380	208	
		During	9.54	5.16	4.38	66	-36	10.41	90.8	A T*	+60	280	186	
		After	9.12	6.21	2.91	100	0	9.56	94.4	N R	60	150	150	
		Before	9.12	6.21	2.91	100	0	9.56	94.4	N R	60	150	150	
		During	9.02	4.35	4.67	62	-38	9.84	90.7	N R	60	280-290	190	

194	14 2	Before	10 25	7 53	2 52	100	-4	11 06	90 1	67 7	N.R.	60	120	200
			10 22	7 61	2 61	96	-5	10 87	93 2	69 6	N.R.		{ 300-110 Av 240	
195	12 4	During	9 91	7 26	2 65	95	-5	10 82	90 8	66 7	N.R.		200	
		After	9 91	7 26	2 65	100	-32	10 82	90 8	66 7	N.R.		200	
		Before	9 84	5 92	3 90	68	-31	10 83	90 0	54 3	A.F.		340-350	
		During	9 11	5 25	3 86	69	-23	10 24	88 1	50 8	N.R.		220	
196	12 4	After	8 67	5 22	3 45	77	-23	9 25	92 8	56 0	N.R.		200	
		Before	12 21	5 90	6 31	100	-8	12 97	93 4	45 2	N.R.		170	
		During	11 62	4 73	6 89	92	-8	12 45	92 6	37 6	N.R.		370-380-390	
197	12 4	Before	10 92	6 89	4 03	100	-20	12 01	90 2	57 0	N.R.		60	
		During	10 85	5 85	5 00	80	+12	11 94	90 1	48 6	A.F.		300-320	
		After	10 60	7 01	3 59	112	+12	11 21	93 8	62 2	N.R.		180	

\* Spontaneous auricular fibrillation was present when this series of observations was taken and its duration was not known. Auricular fibrillation was then continued for one hour and the circulation rate compared with the rate after a period of rest.

*The effect of regular tachycardia on the blood flow* There are 16 observations made on 9 animals. The ventricular rates during the tachycardia varied between 250 and 390 per minute (table 4), the absolute increase in heart rate varying between 30 and 220 per minute and the percentage increase in heart rate varying between 120 and 229 per cent of what they were during the control periods (table 5). In 11 observations in 7 dogs (two thirds of the observations) the blood flow was not altered on changing from the normal rhythm to regular tachycardia (tables 1, 2 and 3) (only changes greater than 10 per cent are considered significant). In 5 observations in 4 dogs (one third of the observations) the blood flow was decreased during the period of tachycardia. Dog 189 falls in both these groups.

TABLE 3  
*Summary of experiments*

Rhythm	Effect on blood flow		Effect on oxygen saturation of arterial blood	
	Decrease	No change	No change	Decrease (4 to 7 per cent)
Auricular fibrillation	10 observa-tions in 10 dogs	0	10 observa-tions in 10 dogs	0
Regular tachycardia	5 observa-tions in 3 dogs	11 observa-tions in 7 dogs	11 observa-tions in 5 dogs	5 observa-tions in 4 dogs

*A comparison of the effect on blood flow of regular tachycardia and auricular fibrillation in the same dog* In 5 dogs we have been able to compare the blood flow during regular tachycardia and during auricular fibrillation of the same ventricular rate (dogs 198 and 197) or at comparable rates (dogs 193, 194 and 195) (table 2). The blood flow in dog 198 was decreased 55 per cent during the period of fibrillation and returned toward normal during the subsequent rest period (fig 2). The blood flow remained unchanged when the heart was driven at the same regular rate. The arterial saturation was unchanged during fibrillation, but was slightly decreased during the period of regular tachycardia. The rhythms which obtained at the time that the blood samples were taken in this dog were recorded electrocardio-

TABLE 4  
*The effect of changes in heart rate on the blood flow in dogs\**

Rhythm	Dog number	Blood flow unchanged		Blood flow decreased	
		Ventricular rate		Ventricular rate	
		During control period	During induced rhythm	During control period	During induced rhythm
Regular tachycardia	189	210	340	160-170	250-280
		210	240-250		
	190	160	300-320		
		190	300-310		
	191	170-180	300-320		
		250	200-310-320		
		160	310		
	196			220	380
				240	350
	198	170	320-330		
Irregular tachycardia	197			180	370-380
				150	280-290
	195	170	370-380-390		
	194	120	110-300		
	193	120	270-280-290		
	151			150-160	48-50†
	157			160-170	330-340
	167			180	270
	169			200-210	350
	193			150	260-370

\* Some of the data on irregular tachycardia are taken from the first paper of this series.

† Femoral rate

graphically (fig 3) In 4 of the 5 dogs there was no change in the blood flow during the period of regular tachycardia, while the blood

TABLE 5  
*The effect of changes in the heart rate on the blood flow*

		Per cent change in heart rate						
		100- 109*	110-129	130-149	150-169	170-189	190-209	210-229
Dogs in which the effect of R and I are compared						197 I -	198 R 0	198 I -
							197 R -	
							197 R -	
						195 I -		193 I -
						195 I -	195 R 0	193 R 0
Dogs R		189 R 0		189 R 0	189 R -		190 R 0	
		191 R 0		190 R 0	191 R 0		191 R 0	
				196 R -	196 R -			
Dogs I						167 I -	157 I -	
						169 I -		
							151 I -†	
Total		0 2 R	-	0 2 R	- 1 R	0 1 R	- 2 R	0 4 R
						5 I		0 2 R
								3 I

In this table the dogs are arranged according to percentage changes in heart rate during the induced rhythm. The corresponding changes in blood flow are indicated by symbols.

Some of the data are taken from the first paper of this series

\* 100 per cent = initial natural rate, R = regular rapid rate, I = irregular rapid rate (i.e., auricular fibrillation), 0 = no change in blood flow, - = decreased blood flow

† Femoral rate, this dog may not be placed in the right column but this has no effect on the conclusions drawn from this table

flow showed the usual decrease during the period of auricular fibrillation. In the fifth dog (dog 197) there was as great a decrease in blood flow during regular tachycardia as there was during the period of

auricular fibrillation, the ventricular rates during the two periods being the same. In dogs 193, 194 and 195, although the absolute

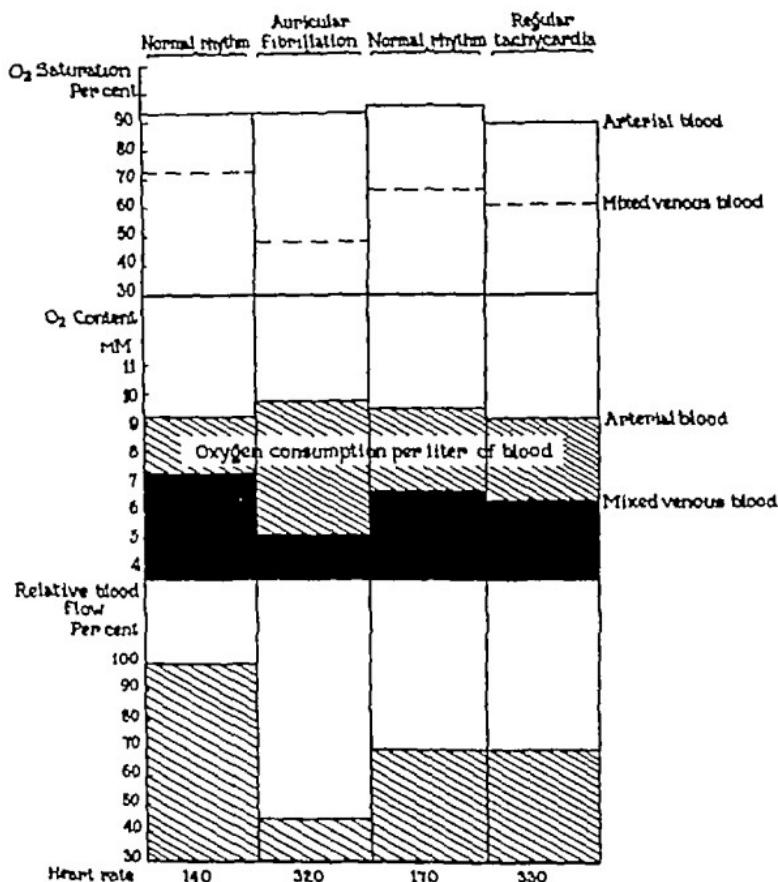


FIG. 2. In this figure is compared the effect of tachycardia both regular and irregular (auricular fibrillation) on the blood flow in dog 198. In estimating the relative blood flows shown in the last three columns, the ratio of the oxygen consumed per liter of blood in each of these periods to the oxygen consumed in the initial normal control period was calculated.

heart rate was slower during the fibrillatory period than during the regular tachycardia (though the percentage increase in heart rate over the control periods were approximately the same (table 5))

there was nevertheless no change in the blood flow during regular tachycardia as against a decrease in the irregular rhythm, emphasizing perhaps more strikingly the difference in effect produced by the two rhythms

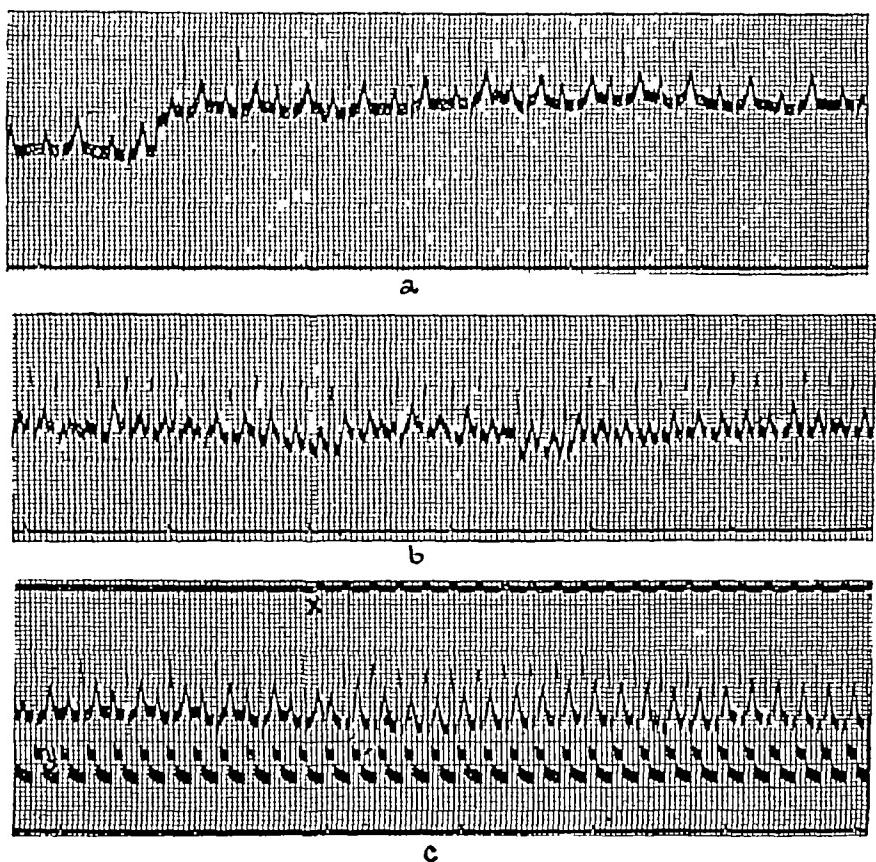


FIG 3 Electrocardiograms (Lead II) are shown obtained from dog 198. 3a was taken during the normal rhythm, 3b during auricular fibrillation and 3c during regular tachycardia. At x the electromagnetic signal shows when the induced break shocks began to operate y is the shadow of the second galvanometer string and indicates at what instant the induced shocks were thrown into the auricle. A short time interval is seen between the electrical stimulus and the auricular P wave which results. Divisions of the ordinates equal  $10^{-4}$  volts. Divisions of the abscissae equal 0.04 of a second. The original curves are sharply contrasted black and white, no half tones are lost by the method of reproduction here used. The curves are reduced to two-thirds of their natural size.

The blood flow was uniformly decreased in auricular fibrillation. In regular tachycardia the blood flow was unchanged in two thirds of the observations and decreased in the other one third.

#### DISCUSSION

Why the blood flow is unchanged in some dogs during regular tachycardia and decreased in others is not clear from the data which we have. It is not due to the absolute increase in heart rate because in dog 189 the blood flow was decreased during a ventricular rate of 250 to 280 per minute, while in dog 195 the heart was driven at a ventricular rate of 390 per minute without a decrease in blood flow occurring (table 4). Neither does it seem to be due to the percentile increase in heart rate. In dog 196 the heart rate during tachycardia was 150 per cent of what it was during the control period and the blood flow was decreased, on the other hand in dog 193 the heart rate was 229 per cent of the rate during the control period without any change in the blood flow (table 5). We have found without exception that the blood flow is decreased in dogs during auricular fibrillation (1) and in this rhythm we know that a pulse deficit occurs in patients. We have raised the question whether a pulse deficit may not occur during experimental auricular fibrillation and also in some animals during regular tachycardia of 250 to 400 per minute. These may be the animals in which a decrease in blood flow has been found. Experiments are now in progress in which we are recording the pulse deficits during these rhythms.

The results of these experiments in dogs parallel the results which Blumgart and Weiss (3) have found in human subjects. They have estimated the circulation time between two points by a new method and have found that it is increased in patients with auricular fibrillation not only when compared to the circulation time in normal subjects, but also when compared to the circulation time in the same patient after the normal rhythm has been restored following the administration of quinidine sulphate.

In two thirds of the observations the arterial oxygen saturation was not affected during regular tachycardia, but in the other one third of the observations the saturation was decreased 4 to 7 per cent (table

3) In auricular fibrillation the arterial oxygen saturation was uniformly unchanged. In none of the observations was the decrease as great as Carter and Stewart (4) and Dieuaide (5) found in their cases of paroxysmal auricular tachycardia and paroxysmal ventricular tachycardia respectively. In these two patients there was disease of the heart muscle as well as of the valves, in these circumstances the reaction to an abnormal rhythm might of course be different from that in presumably normal dogs. That the paroxysm of auricular tachycardia reported by Barcroft, Bock and Roughton (6) occurred in a healthy young student may account for the failure of this patient to show decreased saturation of the arterial blood. Stewart (7) has shown in patients with valvular disease as well as in patients with myocardial disease that the oxygen saturation of the arterial blood is unchanged following the increase in heart rate that occurred after the injection of atropine.

#### SUMMARY

The blood flow has been studied during artificially induced regular tachycardia in normal unanesthetized dogs. It was found that

1 During regular tachycardia the blood flow was usually unchanged, but in one third of the observations it was decreased.

2 During regular tachycardia the oxygen saturation of the arterial blood was usually unchanged, but a small decrease of 4 to 7 per cent occurred in one third of the observations.

3 In 5 experiments the effect of regular tachycardia and auricular fibrillation of the same or comparable ventricular rates was compared in the same dogs. The blood flow was decreased as usual during auricular fibrillation, while in regular tachycardia the blood flow was unchanged except in one observation.

#### CONCLUSIONS

1 The heart is less effective in the propulsion of blood during irregular tachycardia (auricular fibrillation) than during regular tachycardia or the normal slower rhythm.

2 It is possible for the heart to be as efficient in the propulsion of blood during regular tachycardia as during the normal slower rhythm.

3 Tachycardia *per se* does not produce anoxemia of the arterial blood Irregular tachycardia does not affect the oxygen saturation of the arterial blood Regular tachycardia may occasionally be followed by a slight decrease in arterial oxygen saturation

#### BIBLIOGRAPHY

- 1 Stewart, H. J., Crawford, J. H., and Hastings, A. B. *Jour Clin Invest.*, 1926, iii, 435 The Effect of Tachycardia on the Blood Flow in Dogs. I. The Effect of Irregular Rhythms as seen in Auricular Fibrillation
- 2 Cohn, A. E., and Levy, R. L. *Proc Soc Exper Biol. and Med.*, 1922, xix, 174 Experiments with Quinidine on Conduction and on the Refractory Period in the Dog's Heart
- 3 Blumgart, H., and Weiss, S. *Jour Clin Invest.*, 1926, ii, 600 The Velocity of Blood Flow in Health and Disease.
- 4 Carter, E. P., and Stewart, H. J. *Arch Int Med.*, 1923, xxxi, 390 Studies of the Blood Gases in a Case of Paroxysmal Tachycardia
- 5 Dieuaide, F. R. *Johns Hop Hosp Bull.*, 1924, xxxv, 229 Observations on the Respiratory Gases in Ventricular Paroxysmal Tachycardia.
- 6 Barcroft, J., Bock, A. V., and Roughton, F. J. *Heart*, 1921-22, ix, 7 Observations on the Circulation and Respiration in a Case of Paroxysmal Tachycardia.
- 7 Stewart, H. J. *Jour Clin Invest.*, 1926, iii, 241 The Effect of Increased Heart Rate Due to the Injection of Atropine on the Oxygen Saturation of the Arterial and Venous Blood of Patients with Heart Disease



## EPIDEMIC PREVALENCE IN THE LIGHT OF EXPERIMENTAL FINDINGS

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This is perhaps a timely occasion to classify and evaluate the results of seven years' experimental study of epidemic diseases. At the annual meetings of this association in 1921, Flexner (1) outlined a plan for the investigation of mouse typhoid infection under the controlled conditions of the laboratory, previously, Topley (2), in London, delivered the Goulstonian lectures on the same subject. Since then, the English investigators, the group at the Rockefeller Institute in New York, and Neufeld and Lange, in Germany, have furthered these studies and brought them to a state suitable for discussion. Hence, although admirable critiques have already been published recently by Neufeld (3), Topley (4), and Flexner (5), it may be of use to summarize briefly our own conclusions, and relate them as far as possible to epidemic prevalence in man.

Furthermore, there is special need at present for a restatement of the general problem, a classification of current theories, and formation of a basis for further investigation. This implies a review of methods and techniques employed, with a discussion of the mutual relationship, advantages, and limitations of each. To these questions, therefore, it is proposed to give attention as well as to summarize briefly the actual results obtained.

The first step in the experimental study of an epidemic disease is the close observation of the infection as it occurs naturally. Knowledge of the distribution of the specific microbes in nature, their portal of entry into the animal body, various types of animal response, etc., is essential. Thus prepared, one is in a position to plan experiments under conditions which are controlled and yet simulate nature, and to attain results which are relatively free of a certain inevitable labora-

tory error, and which may be checked against the observed phenomena of the spontaneous disease

In this connection, two native diseases have been studied mouse typhoid, by Lynch and Amoss (6), and later by ourselves (7), and rabbit lepisepticum infection (7). The former is known to be of intestinal origin, the latter respiratory. Both are widespread. Both may persist mildly in a given community, or may assume epidemic proportions with or without apparent periodicity, both prevail more frequently in spring and fall and affect certain individuals and races more severely than others. In short, the two animal diseases present for solution the problems common to human epidemiology.

The second step consists in inducing the various interepidemic and epidemic phases of the diseases in the laboratory under controlled conditions. Both Topley (8) and Amoss (9) have done this successfully with the aertrycke (animal paratyphoid B) mouse typhoid, Topley likewise (10) with mouse pasteurella, and Webster with the *B. enteritidis* mouse typhoid (7) and rabbit lepisepticum infection (7). Topley placed mice in a large cage, introduced the specific bacilli by carriers or contaminated food, and added fresh mice to the population at stated intervals. Amoss placed small numbers of mice in boxes and allowed the attendant to spread the infection from one focus by feeding and cage cleaning manipulations. We have used Topley's method in our mouse typhoid and a modification of Amoss' in our rabbit lepisepticum experiments.

This series of experiments, besides proving that epidemics, resembling closely those occurring "spontaneously," may be induced at will, brought out several other facts of importance. Briefly, they are as follows: (a) that a single focus of mouse typhoid in a community is followed by widely scattered sporadic cases (11), (b) that the general mortality increases during the pre-epidemic period (12), (c) that the addition of fresh individuals into an infected community revives the epidemic (8, 9), (d) that the epidemic peak may be maintained by daily replacements of dead with fresh mice (8), (e) that survivors may or may not be carriers, or be immune to subsequent infection (13, 9), and (f) that the height of the epidemic waves and intervals between them is affected by the rate of immigration of fresh mice,—the greater the immigration rate, the greater is the mortality,

the less pronounced the epidemic wave, and the less apparent the interval between them (10) Thus it was observed that the experimental epidemics reproduce the important phenomena observed in human epidemics and afford an unusual opportunity for analysis

To explain the mode of spread of the epidemics engendered in the laboratory has, therefore, become the task of the experimental epidemiologist As his material he has a disease which behaves as human infections do, and which he may use as he sees fit for the solving of the problem He may regard the phenomena as the result of a mass of indeterminable variables, and hence be forced to apply statistical methods of analysis He becomes aware that this procedure in no way proves the truth or falsity of any theory, but merely simplifies its statement. In this way he analyzes the data precisely as one would corresponding human data, and goes no further in solving the essential problem Such an analysis has recently been carried out by Topley (4) On the other hand, he may pursue the experimental method, determine by quantitative measurement the various factors which control the spread of the disease, and determine, as far as may be, their values and mutual relationships It is this latter method which especially has engaged our attention (14) and hence we shall describe briefly its employment and the results attained

The three factors responsible for the spread of infection, that is host susceptibility, microbic dosage, and microbic virulence, are like all biological phenomena, exceedingly difficult to measure accurately Each is known to be the resultant of a number of variables, of which some are indeterminable Host susceptibility must be regarded as the sum of the variables which influence the response of the animal to bacteria or other injurious agencies, microbic virulence, the total of the variables which determine their pathogenic power, and microbic dosage, the united variables which decide the number of organisms available to the host *A priori*, none of the factors can be regarded as a constant and no one may be used uncontrolled as a standard of measurement, as, for instance, host susceptibility and duration of life as measures of bacterial virulence, in short, no uncontrolled response to a given injury may be considered an accurate measurement

It is essential, therefore, to reduce as far as possible the number of variables contained in each of the three factors This has been accom-

plished as follows Dosage has been rigidly controlled by administering to each animal a definite number of organisms by way of the normal portal of entry, thus insuring each animal being exposed in the natural way to a quantitatively known dose Virulence has been controlled, as far as possible, by keeping bacterial cultures on constant media at 4°C , and using for the inoculation a single culture, freshly grown in a standard fluid medium Finally, certain variables contained in the host have been eliminated, namely, those due to (a) heredity, by using only pure line strains of mice, inbred for five years or more, (b) environmental factors, by breeding and raising the mice in a special room, where temperature, food, cage cleaning, space, etc , are standardized Possible differences, due to (c) age and weight, are removed by using young adults ten to twelve weeks old, weighing 18 to 20 grams, and finally, variations resulting from (d) acquired, specific resistance are eliminated by keeping the room entirely free from disease Each mouse, therefore, is known to have had no previous exposure to the organisms Finally to secure average uniform conditions, 50 to 100 mice are generally employed for each test

The extent to which control of these factors had been achieved was determined by comparing the mortality rates of different groups of mice inoculated simultaneously with definite numbers of a common strain of mouse typhoid bacilli If sufficient of the variables had been eliminated, the mortality rates of any number of mouse groups should be the same And in fact, they proved uniform for each group the average difference in total mortality among groups of fifty mice was less than 1 per cent per day throughout the sixty day period of observation, of groups of twenty-five, not more than 5 per cent, and of groups of twenty, about 10 per cent (14 and 7) These results show that a significant number of variables had been eliminated from the factors and each factor, under the conditions of the experiment and at a given time, was relatively constant The mortality curve attained measured quantitatively the reaction between host and microbe and could be considered as a standard It has been designated, therefore, the "standard control curve" and forms the basis of all our subsequent work (14)

Only with some such standard of measurement is an analysis of

epidemic phenomena made possible, and only by controlling the factors in some such way as we have indicated is one able to obtain such a standard. It is not surprising therefore that Topley, who used mice purchased from dealers, of mixed race, unknown age and environmental circumstances, including previous exposure to infection, has not been able to produce a standard of measurement. His attempts have resulted in widely scattered curves, described by him as totally random. Consequently, his epidemiological experiments contain no satisfactory measurements of the several factors involved and he is driven as in human epidemiological observations to the employment of statistical analyses of the unknown and indeterminable variables. All titration of bacterial virulence must be made against some known standard, where dosage and host factors are known to be constant. The application of this principle is universal throughout the field of bacterial experimentation.

Measurement of the factors concerned in the spread of mouse typhoid and rabbit lepisepticum infection having been carried out in the manner described above, the results attained have been described (14) as follows. Concerning host susceptibility, "we have found that this quality of resistance is present in different amounts in individuals of the same family or race, and that differences, under properly controlled conditions, take the form of a frequency curve. Furthermore, by an artificial selection of especially resistant or susceptible individuals it has been possible to breed strains at will, whose average resistance is greater or less than that of the original random group. It seems probable, then, that successive descendants of two given individuals inherit definite amounts of potential resistance, which vary according to the law of probability about a mean which approximates the mean resistance of the original pair. It would seem that racial differences in resistance to mouse typhoid infection can be expressed by a relatively constant value. And finally, whatever the potential inherited constitution of individuals, families, or races may be, it is affected profoundly by seasonal influences, food, and general hygienic conditions. For this reason it is erroneous to speak of the presence or absence of resistance as though it were a unit factor. Rather it should be considered a manifestation of an extremely complex mechanism,

modified by heredity and environment, whose quantity in individuals of a large group tends to follow the laws of chance, about a mean which is more or less a characteristic of the race" (15)

The virulence of type pure strains of mouse typhoid bacilli and *Bact. lepisepticum* has proved to be constant under conditions comparable to those which occur in nature. There is no geometric rise in virulence before and during an epidemic wave and no corresponding drop at the peak and during its decline. Type pure strains apparently conform to general biological rule and maintain a uniform degree of pathogenicity. It is true that bacterial variation does occur, and that the changed forms are less virulent. Thus, *Bact. lepisepticum* in the presence of oxygen at atmospheric pressure yields variants of low pathogenicity which do not revert to the original form. And mouse typhoid bacilli, in the presence of relatively large amounts of bacteriophage, or other injurious agencies, yield "rough" or "mucoid" colonies, which also are less virulent. But as far as observations of the natural and laboratory diseases have gone there is no indication whatever that these deteriorations play a significant part in the major phenomena of epidemics. The present conclusion, therefore, is that the microbial virulence factor, in so far as the spread of disease is concerned, constitutes a constant.

Microbic dosage, on the contrary, has been found to exert a highly important influence. When the dose is below a certain critical level, the resulting mortality curve is low and irregular; when above, it takes on the characteristic form and is changed but little whether hundreds or thousands or millions of bacilli are available. Furthermore, a critical dose or more of mouse typhoid bacilli given to the Rockefeller Institute strain of mice yields a mortality curve which may be superimposed upon the experimental epidemic curves recorded by Amoss. In keeping with this the demonstration has been made experimentally that in course of mouse typhoid epidemics the number of bacilli available to a given population increases from a negligible quantity to several millions six to eight days before the onset of an epidemic wave, and drops below the critical point a few days preceding the decline in mortality (14).

The bearing of these various observations and measurements on the theory of epidemic prevalence is considerable. Among other

things it becomes necessary to substitute for "change in virulence" the notion of "change in host susceptibility and dosage." The experimental data provide support for certain observations on differences in individual resistance which clinicians have long held as self evident, and suggest an explanation as to the manner of spread of diseases such as exanthemata, influenza, and pneumonias, less well understood than those we have described.

Three hypothetical states of equilibrium of any population in respect to a given microbe may be recognized, the community may be entirely free, it may harbor the more saprophytic forms of low virulence, or it may contain scattered foci of the virulent forms. In the first instance, an epidemic arises by a widespread distribution of virulent organisms coming from without, in the second, possibly by a widespread dissociation or mutation and distribution of already present, non-virulent types to virulent forms, and in the third, by dissemination of already present virulent forms throughout the population. In typhoid-dysentery outbreaks, due to food or water contamination, in influenza epidemics, primary plague, cholera, and syphilis epidemics such as prevailed in western Europe during the thirteenth, fourteenth, and fifteenth centuries, the virulent microorganisms were certainly introduced from without. Whereas in the urban typhoid-dysentery out-breaks which still sometimes arise, in present-day pneumonias, exanthemata, common colds, oriental plague, and cholera, the inciting virulent microorganisms are undoubtedly already present in the given community. There is no present evidence indicating that saprophytic microorganisms of low virulence present in a community undergo an increase in pathogenicity or mutate, become widespread and excite epidemics. Virulent or so-called "epidemic" strains of microorganisms must, therefore, either be introduced into a community from without, or be already present in scattered foci.

The mechanism underlying microbial distribution has still to be explained. Probably population susceptibility plays a large part. Extraneous microbes introduced into a virgin population find a large proportion of susceptibles. In them, they multiply rapidly and thus become widespread. Microbes already present in a community must likewise increase in numbers in the tissues of susceptibles. Measles becomes epidemic every second or third year in New York,

as the number of susceptibles increases above the critical level, pneumonia increases in the spring and fall at the time when deaths from all causes increase and susceptibility seems greatest, oriental plague and cholera, war-time epidemics occurring at periods of stress and strain on population resistance, all suggest some relationship between altered resistance and increase in dosage

However this may be there is no doubt that adequate available dosage is the essential incitant of an epidemic, and experiment has shown that the actual curve, morbidity or mortality rate, is an expression of the resistance, hereditary and acquired, specific and non-specific, of the population group exposed to a given dose of pathogenic microbes. When microbial distribution has been sudden and widespread throughout a susceptible population, a simple left skew frequency curve results, when dosage is intermittent, plateaus and multiple peaks occur, and when population resistance is high, the curve becomes gradual and low. Morbidity or mortality rates describe actual host susceptibility to a given dose of microbes and not fanciful fluctuations in microbial virulence.

#### BIBLIOGRAPHY

- 1 Flexner, S., and Amoss, H. L. Trans Assoc Amer Phys, 1921, xxvi, 34  
A Physical Basis for Epidemiology
- 2 Topley, W. W. C. Lancet, 1919, ii, 1, 45 and 91 Spread of Bacterial Infection (Goulstonian Lectures)
- 3 His, W. Deut Med Wchnschr, 1925, li, 299 Seuchenprobleme I Einleitende Worte  
Neufeld, F. Deut Med Wchnschr, 1925, li, 341 Seuchenprobleme II Neue Ergebnisse der Experimentellen Forschung
- 4 Topley, W. W. C. Lancet, 1926, i, 477, 531 and 645 Experimental Epidemiology (Milroy Lectures)
- 5 Flexner, S. Amer Jour Med Sci, 1926, clxvi, 469 and 625 The Advancement of Epidemiology Through Experiment I and II
- 6 Lynch, C. J. Jour Exp Med, 1922, xxvi, 15 An Outbreak of Mouse Typhoid and Its Attempted Control by Vaccination
- 7 Webster, L. T. To be published shortly
- 8 Topley, W. W. C. Jour Hyg, 1921, xix, 350 The Spread of Bacterial Infection Some Characteristics of Long-Continued Epidemics
- 9 Amoss, H. A. Jour Exp Med, 1922, xxxvi, 45 Experimental Epidemiology II Effect of the Addition of Healthy Mice to a Population Suffering from Mouse Typhoid

- 10 Greenwood, M., and Topley W W C *Jour Hyg*, 1925 xxiv, 45 A  
Further Contribution to the Experimental Study of Epidemiology
- 11 Amoss, H L *Jour Exp Med*, 1922, xxxvi 25 Experimental Epidemiology  
I An Artificially Induced Epidemic of Mouse Typhoid
- 12 Topley W W C *Jour Hyg*, 1922, xxi, 10 The Spread of Bacterial  
Infection Some Characteristics of the Pre Epidemic Phase
- 13 Topley, W W C *Jour Hyg*, 1921, xx, 103 The Spread of Bacterial In  
fection The Potential Infectivity of a Surviving Mouse Population and  
Their Resistance to Subsequent Epidemics of the Same Disease
- 14 Webster L T *Jour Exp Med*, 1923, xxxvii, 231 Microbic Virulence  
and Host Susceptibility in Mouse Typhoid Infection  
Webster, L T *Jour Exp Med*, 1923, xxxvii, 269 Contribution to the  
Manner of Spread of Mouse Typhoid  
Webster L T *Jour Exp Med*, 1923, xxxvii, 781 The Virulence of an  
Epidemic Strain of *Bacillus Pestis Cavae*  
Webster, L T *Jour Exp Med*, 1923, xxviii, 35 and 45, 1924, xxxix 129 and  
879 Microbic Virulence and Host Susceptibility in Parathyroid Enteritidis  
Infection of White Mice. I, II III and IV
- Webster L T *Jour Exp Med*, 1924 xxxix, 837, 843 and 857, 1924, xl,  
109 and 117 The Epidemiology of a Rabbit Respiratory Infection I, II,  
III IV and V
- Webster, L T and Pritchett I W *Jour Exp Med*, 1924, xl 397 Microbic  
Virulence and Host Susceptibility in Parathyroid Enteritidis Infection  
of White Mice V
- Smith D T, and Webster, L T *Jour Exp Med*, 1925, xli, 275 Epidemiological  
Studies on Respiratory Infections of the Rabbit VI Etiology  
of Otitis Media.
- Webster, L T *Jour Exp Med*, 1925, xli, 571 Biology of *Bacterium Lepi*  
*septicum* I
- Pritchett, I W *Jour Exp Med*, 1925, xli, 195 and 209 Microbic Virulence  
and Host Susceptibility in Parathyroid Enteritidis Infection of White  
Mice. VI and VII
- Webster, L T, and Baudisch, O *Jour Exp Med*, 1925, xlii, 473 Biology  
of *Bacterium Lepisepticum* II
- Webster, L T *Jour Exp Med*, 1926, xlili, 555 and 573 Epidemiological  
Studies on Respiratory Infections of the Rabbit VII and VIII
- Pritchett, I W *Jour Exp Med*, 1926, xlili 143, 161 and 173 Microbic  
Virulence and Host Susceptibility in Parathyroid Enteritidis Infection of  
White Mice IX, X and XI
- Webster, L T *Amer Jour Hyg*, 1924 iv, 134 The Application of Ex  
perimental Methods to Epidemiology
- Webster L T *Amer Jour Hyg*, 1925, v 335 Further Contributions of  
Experimental Methods to the Study of Epidemics
- 15 Webster, L T *Jour Exp Med* 1925 xlvi, 1 Microbic Virulence and  
Host Susceptibility in Parathyroid Enteritidis Infection of White Mice.  
VIII



# A TECHNIQUE FOR MEASURING X-RAY PHOTOGRAPHS OF THE CARDIAC AREAS OF DOGS

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It is often important in the study of problems of the circulation to be able to determine what effect an experimental procedure has on the size of the heart of the animal under investigation. For this reason we have developed and adapted a technique for obtaining x-ray photographs of the dog's heart, so that successive records are comparable as to the size of the shadow cast by the heart. The method for obtaining the area of the heart shadow is the one commonly in use as devised by Levy (1).

## TECHNIQUE

*Position of the dog.* The exposures are made in the antero-posterior position with the dog horizontal. The dog lies on an animal board especially constructed for this purpose. That portion of the board, about 20 by 20 inches, which corresponds to the portion of the dog's chest, is cut out and replaced by a thin sheet of aluminum, below which the cassette carrying the x ray film is placed. The aluminum is used because it cuts out fewer rays than the wood. On each side of the animal board at the level of the chest a right angle upright which is adjustable is attached in order to support the dog. After the dog has been placed on the board straight and without rotation, the uprights can be brought close to the dog's chest and screwed in place (fig. 1). It is necessary to hold the dog's head in place.

*X-ray technique.* The plates are taken at a distance of 2 meters, with an exposure of, on the average,  $\frac{1}{2}$  second. Dogs weighing more than 18 kgm require a slightly longer exposure. The exposure is made at the height of inspiration, the short exposure time being necessary because the dogs breathe rapidly. To obtain sharp pictures

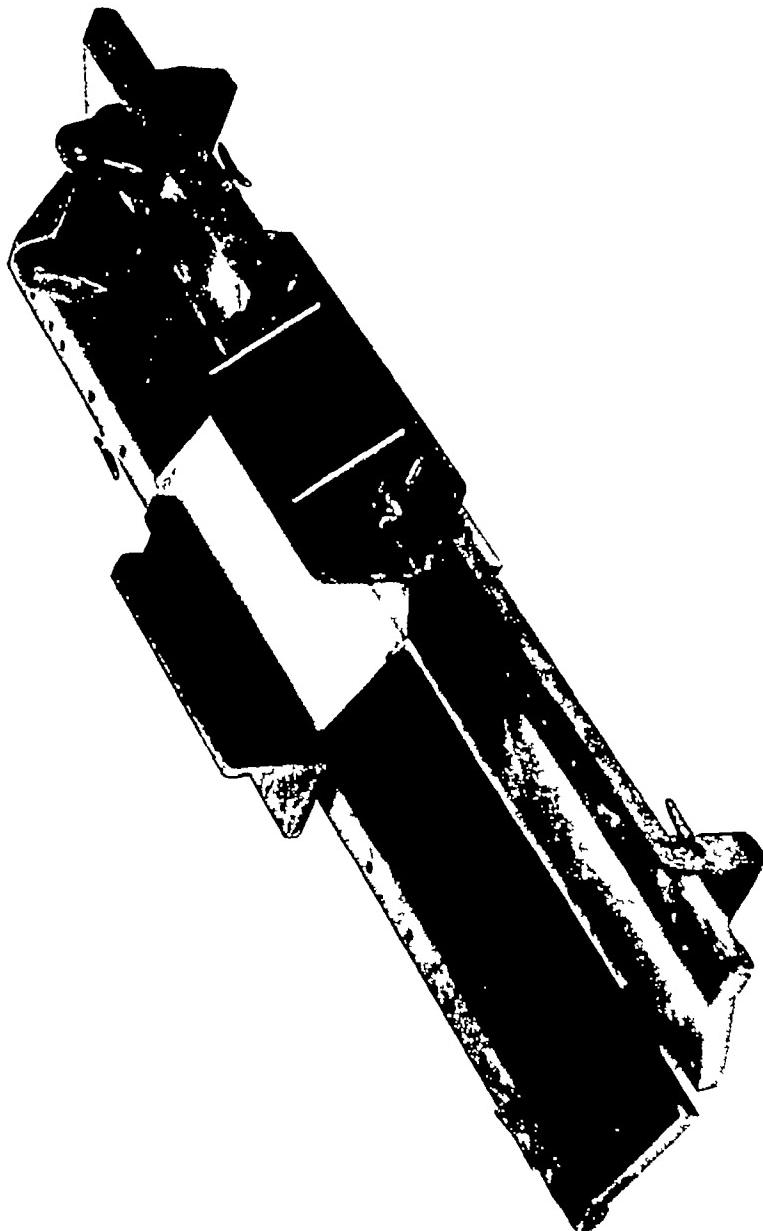


FIG 1 PHOTOGRAPH OF ANIMAL BOARD, SHOWING ADJUSTABLE UPRIGHTS FOR HOLDING THE DOG IN POSITION

with this short exposure time, we use a spark gap of 9 inches and a 50 milliampere current, with super speed film<sup>1</sup>. This technique gives

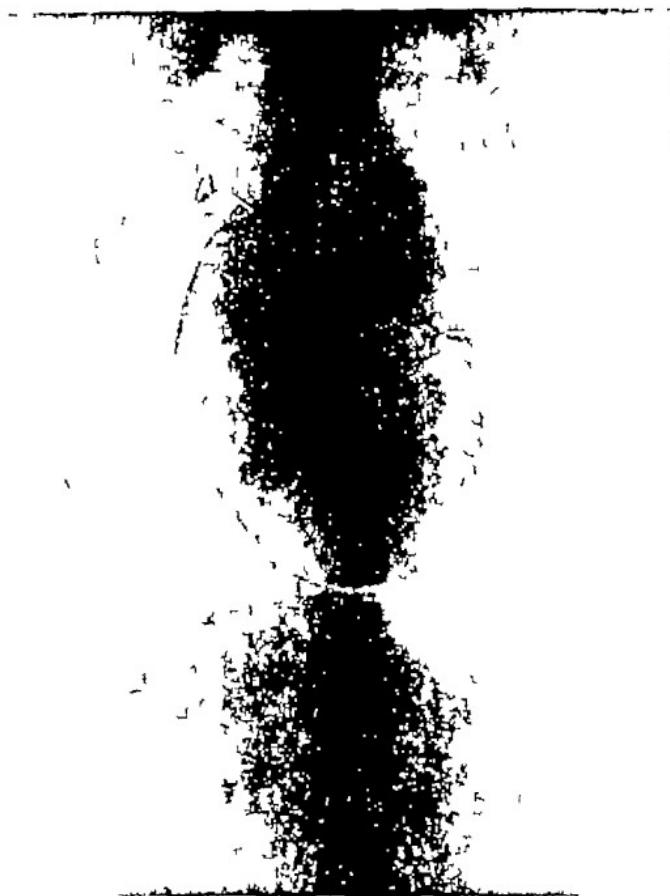


FIG 2 (REDUCED TO ONE HALF) X RAY OF DOG'S HEART OBTAINED BY THE AUTHOR'S TECHNIQUE SHOWING POINTS A AND B AND THE HEART OUTLINED WITH A SOFT CRAYON

a sharp definition of the heart shadow without blurring of the rib shadows, which is so often seen when dogs breathe during the exposure.

*Measurement of the heart area* In the photograph the apex of the

<sup>1</sup> Eastman superspeed duplitized films used with French screens in cassettes

heart is usually found a little above the diaphragm or at most barely touches it, so that this part of the heart's contour and the ventricular borders can be easily outlined. It is necessary however to complete the contour of the heart at the base, in order to do this two points are located. On the right, one point is found where the shadow of the right auricle passes into the shadow of the great vessels, this is point (*A*) (fig 2). On the left there is usually an indentation where the left ventricular shadow passes into the left auricular shadow, and this is the second point (*B*). Beginning at (*A*) the heart shadow is outlined on the film with a soft crayon down to the apex and from the apex up to (*B*). These lines are then traced on a sheet of paper. Between the points (*A*) and (*B*) the outline is completed in the following manner. With compasses the distance between points (*A*) and (*B*) is measured, and with this distance as a radius and with (*A*) as the centre, an arc of a circle is described, and then with (*B*) as a centre and the same radius, another arc is described intersecting the first arc. With the point of intersection of these two arcs as the centre and the same radius, an arc is described between (*A*) and (*B*), completing the heart outline (Levy). The area so formed includes the right ventricle, left ventricle, part of the right auricle and part of the left auricle. Since the area measured in this way is used as the basis of comparison with the area of the heart's shadow at a later time it is necessary only that the area measured includes the same portion of the heart. The area outlined is measured with a planimeter,<sup>2</sup> two tracings being made, the two readings may differ by not more than 0.5 sq cm.

*Protocols* Two types of experiments were carried out. In the first the dog was removed from the board after the photograph was taken and was then put back, when a second one was made. It was found in four experiments that the greatest variation from the first

<sup>2</sup> A convenient method of quickly measuring the heart area is as follows. An unexposed x-ray film, 14 by 17 inches, is developed washed and dried. The four corners are then fixed to a drawing board with thumb tacks. The sheet of paper with the tracing of the heart is slipped under the cleared film and the heart outline measured with a planimeter. The wheel of the planimeter glides easily over the surface of the film.

record in a large number of photographs was less than 3 per cent (table 1)

In the second type of experiment the variation in the measurements of the heart area over a more prolonged period of time was ascertained. Four normal dogs were x-rayed every day for 8 days and the heart areas measured. In addition to this observation, the dog's weights were recorded (table 2). The heart area of the first record of each dog was represented as 100 per cent and the succeeding heart areas were calculated as percentages of this area. The weights were treated in a similar way. The greatest variation in heart area was found in dog 49 on the seventh day, when the heart area was 104.9

TABLE 1

*Heart areas of normal dogs with a short time interval between successive photographs*

Dog number	Date	Area sq cm	Per cent of first area per cent	Variation per cent
71	October 25	54.25	100.0	0.6
		53.95	99.4	
75	October 26	54.65	100.0	1.5
		55.60	101.5	
76	October 27	52.50	100.0	1.0
		52.00	99.0	
77	November 6	55.75	100.0	2.1
		56.93	102.1	

per cent (table 2) of its original area, with a variation of 4.9 per cent. Dog 66 showed a negative variation of 4.5 per cent. The total variation in dog 66 was 5.5 per cent (between 95.5 and 101.0 per cent), and in dog 49 it was 5.3 per cent (between 99.6 and 104.9 per cent), and in dogs 67 and 75 the variation was even smaller. In figure 3 we have plotted the heart area and body weight percentages which are tabulated (columns 4 and 6, table 2) to show graphically the variation for each of the 4 dogs. In figure 4 the heart area percentages of the 4 dogs are combined in a single chart to show the grouping of the points about the base line. The body weights are treated in a

similar manner. The greatest variation seen was 5 per cent greater or 5 per cent less than the original heart area, and although in any

TABLE 2  
*Heart areas and weights of normal dogs over a period of 8 days*

Dog number	Date	Weight kilos	Per cent of first weight given	Heart area sq cm	Per cent of first heart area	Difference between highest and lowest heart area per cent
49	1923					
	May 11	18 28	100 0	63 65	100 0	
	May 12	18 33	100 3	65 40	102 7	
	May 14	18 00	98 5	64 55	101 4	
	May 15	18 75	102 6	63 30	99 6	5 3
	May 16	18 00	98 5	65 55	102 9	
	May 17	17 90	97 9	66 80	104 9	
66	May 18	17 75	97 1	64 70	101 6	
	May 11	18 50	100 0	60 18	100 0	
	May 12	18 00	93 7	60 68	100 8	
	May 14	17 80	96 1	59 00	98 0	
	May 15	18 55	100 2	60 30	100 1	5 5
	May 16	17 50	94 6	57 50	95 5	
	May 17	17 75	95 9	60 90	101 0	
67	May 18	17 30	93 5	60 58	100 6	
	May 11	17 28	100 0	48 75	100 0	
	May 12	17 05	98 7	48 50	99 5	
	May 14	16 80	97 2	48 40	99 2	
	May 15	17 75	102 7	50 50	103 6	
	May 16	17 30	100 1	50 15	102 8	4 6
	May 17	17 15	99 2	50 20	102 9	
75	May 18	17 05	98 7	50 60	103 8	
				50 15	102 8	
	May 11	19 08	100 0	60 30	100 0	
	May 12	18 78	98 4	60 50	100 3	
	May 14	18 75	98 3	59 63	98 8	
	May 15	19 20	100 6	62 60	103 6	
	May 16	18 50	96 9	60 50	100 3	4 9
	May 17	18 55	97 2	60 45	100 2	
	May 18	18 50	96 9	62 58	103 7	
				62 33	103 3	

one dog the variation did not extend to this maximum figure in both directions we have accepted a range of 10 per cent as the normal

variation, thus including the normal variation in the size of the heart and the errors involved in getting the dogs in the same position and in measuring the area with the planimeter. This is the same variation

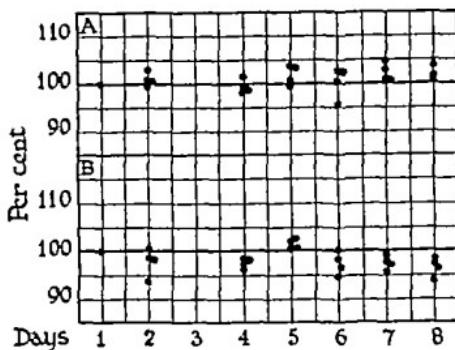


FIG. 3 PERCENTAGE CHANGES IN HEART AREA AND BODY WEIGHT OF 4 NORMAL DOGS OVER A PERIOD OF 8 DAYS

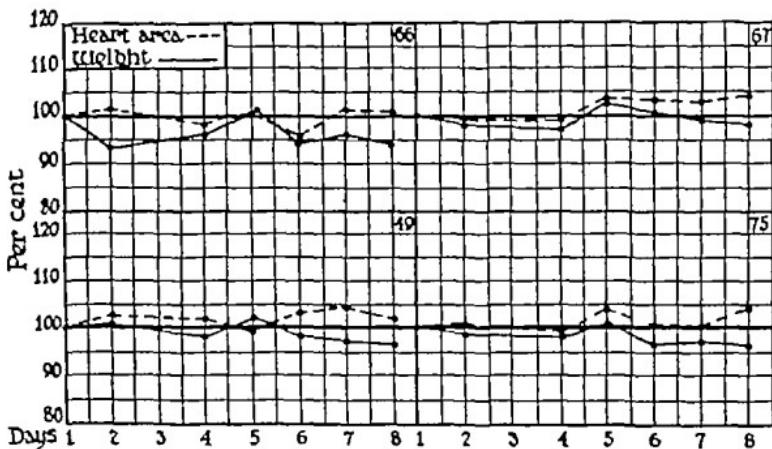


FIG. 4 PERCENTAGE CHANGES OF 4 NORMAL DOGS COMBINED INTO ONE CHART TO SHOW THE DEVIATION FROM THE INITIAL BODY WEIGHTS (B) AND HEART AREAS (A) WHICH ARE REPRESENTED AS 100 PER CENT

which Levy found in measuring the shadows of normal human hearts. During the period of observations there was no significant change in the weights of the dogs.

## SUMMARY

- 1 A technique is described for obtaining and measuring x-ray photographs of dog hearts
- 2 A variation greater than 10 per cent was not obtained in normal dogs over a period of 8 days A variation greater than this can be interpreted as a real change in the size of the heart
- 3 The variation that occurred in photographs made on the same day was less than 3 per cent

## BIBLIOGRAPHY

- 1 Levy, R L Arch Int Med , 1923, vxxii, 359 The Size of the Heart in Pneumonia

# THE EFFECT OF REGULAR AND IRREGULAR TACHYCARDIAS ON THE SIZE OF THE HEART

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This study is concerned with the effect of tachycardia on the size of the heart in normal unanesthetized dogs

Meek (1) has studied the effect of changes in pulse rate on the diastolic size of the x-ray shadow of the heart. In unanesthetized dogs he obtained slow rates by the administration of morphine, followed by the injection of atropine to obtain the more rapid rates. Other dogs were given ether anesthesia and the variations in heart rate obtained by electrical stimulation of one of the vagi after double vagotomy. He found that the size of the heart decreased when the heart rate was increased. He did not obtain rates beyond 200 per minute. He thought that the effect which he observed was due to falling venous pressure, for it did not occur if the venous pressure was maintained artificially by the injection of saline-acacia solution into the left jugular vein. Hodges and Eyster (2) made x-ray photographs of the hearts of patients during periods when the rate was slow and at intervals following the injection of atropine. They were able in this way to compare the effect of rates between 60 and 130 per minute. They found that the size of the heart remained fairly constant for slight variations in pulse rate. The higher rates were accompanied by slight decrease in size of the heart.

The following studies differ from those reported by Meek in that we have been able in the first place to study the effect of tachycardia in normal unanesthetized dogs without the use of drugs to obtain changes in heart rate, in the second place we have been able to obtain very rapid rates, and in the third place we have been able to compare the effect of regular and irregular tachycardia (auricular fibrillation) on the size of the heart in the same dog.

## TECHNIQUE

The detailed method of preparing the dogs for these experiments has been described in a preceding paper (3). With sterile operative technique two wire electrodes were sewed to the right auricles. Intratracheal ether anesthesia was used. The day following this preliminary operative procedure the heart was stimulated through the electrodes and the effect of regular and irregular tachycardia on the blood flow was observed (3) (4). On the next day the effect of these rhythms on the size of the heart was investigated by means of x-ray photographs of the heart.

The x-ray photographs were made according to the method described by Stewart (5) for obtaining photographs of the hearts of dogs under uniform conditions. The anticathode was placed at a distance of 2 meters from the photographic film. The dogs lay quietly on the animal board without anesthesia throughout the period of stimulation. A small needle was inserted in the skin in the midline of the anterior chest wall at the level of the heart. The anticathode was always centered on this same point before plates were exposed in case the table had shifted or the dog had moved during the experiment. Three x-ray photographs were made during each experiment: the first, during the normal rhythm; the second, after the induced rhythm had been present for 1 hour and while it was still present; and the third, a few minutes after the stimulation was discontinued. Faradic current derived from 1 to 3 dry cell batteries in the primary circuit of a Du Bois-Reymond induction coil was used to induce auricular fibrillation. Single induced break shocks thrown into the right auricle at a rapid regular rate (250 to 390 per minute) were used to induce regular tachycardia. The mechanism by which this was done has been described in a preceding paper (4). The stimuli from the apparatus in the physiological laboratory were carried to the x-ray laboratory by insulated wires running in well grounded pipes through the walls of the building. The speed at which the heart was driven in the periods of regular tachycardia was usually the same as that at which it was driven when the blood flow was studied on the preceding day. When observations were made both on the effect of regular and of irregular tachycardia in the same dog one set of observations was made during the morning and the second set in the afternoon after a rest period of 2 to 3 hours.

The method of tracing the x-ray shadows of the hearts and measuring them was that described by Stewart (5). The error of the method is less than 3 per cent, only changes greater than this are significant.

## RESULTS

*The effect of regular tachycardia on the size of the heart.* In 4 dogs we have observed the effect of regular tachycardia on the size of the heart. The natural rates for these dogs varied between 170 and 180

per minute, although in one it was 200 per minute. The rates during artificial tachycardia were 300 per minute. The size of the heart was decreased during the period of tachycardia in all of the observations. The decrease varied between 7 and 14 per cent (table 1). In two dogs, nos 193 and 194, further decreases were found to occur when x-rays were taken a few minutes after the stimuli had been discontinued. In dog 194 the heart had returned to its normal size 2 hours after the stimulation had been stopped.

TABLE 1  
*The effect of regular tachycardia on the size of the heart*

Dog number	Rhythm	Heart rate per minute	Heart area	Heart area per cent of initial	Change in area	Summary of effect
190	Normal	180-190	57.6	100		
	Regular tachycardia	300	49.8	86	-14	Decrease
191	Normal	170-180	45.4	100		
	Regular tachycardia	300	42.2	93	-7	Decrease
193	Normal	170	75.6	100		
	Regular tachycardia	280-290	65.4	86	-14	Decrease
	Normal		61.5*	81	-19	
194	Normal	200	46.2	100		
	Regular tachycardia	300	42.6	92	-8	Decrease
	Normal		41.5*	89	-11	
	Normal		46.2†	100		

\* This x-ray was made a few minutes after stimulation was discontinued.

† This x-ray was made 2 hours after the end of the stimulation period.

*A comparison of the effect of regular and irregular tachycardia on the size of the heart.* In 3 dogs we were able to compare in each case the effect of regular and irregular tachycardia on the size of the heart. During regular tachycardia the size of the heart decreased from 10 to 14 per cent (table 2). During auricular fibrillation the size of the heart was unchanged in two observations and was increased 11 per cent in the third. In dog 189 the size of the heart was 13 per cent less during the period of regular tachycardia (rate 300 per minute) than

it was during that of the normal rhythm (rate 160 to 170 per minute) (figs 1, 2a and 2b) On a second occasion it decreased 14 per cent during the period of regular tachycardia During the period of auricular fibrillation there was no alteration in the size of the heart

TABLE 2

*A comparison of the effect of regular and irregular tachycardia (auricular fibrillation) on the size of the heart in the same dog*

Dog number	Rhythm	Heart rate per minute	Heart area sq cm	Heart area per cent of initial per cent	Change in area per cent	Summary of effect
189	Normal	160-170	33.8	100		
	Regular tachycardia	300	29.4	87	-13	Decrease
	Normal	210	33.3	100		
	Regular tachycardia	340	28.6	86	-14	Decrease
	Normal		26.6*	80	-20	
	Normal	160-170	32.4	100		
195	Auricular fibrillation	300	32.0	100	0	No change
	Normal	170-180	48.3	100		
	Regular tachycardia	380-390	43.7	90	-10	Decrease
	Normal		44.8*	92	-8	
	Normal	170-180	40.7	100		
	Auricular fibrillation	300-320	45.0	111	+11	Increase
198	Normal		48.5*	119	+19	Increase
	Regular tachycardia	170-180	55.4	100		
	Normal	300	49.4	89	-11	Decrease
	Normal		45.3*	82	-18	
	Auricular fibrillation	170-180	55.2	100		
	Normal	300	54.5	99	-1	No change
	Normal		52.6*	95	-5	

\* This x-ray exposure was made a few minutes after the stimuli had been discontinued

although the heart rate increased from 160 per minute to 300 per minute (figs 1, 3a and 3b) In dogs 189 and 198 the tendency of the heart to decrease in size further after the discontinuance of the stimuli is again seen

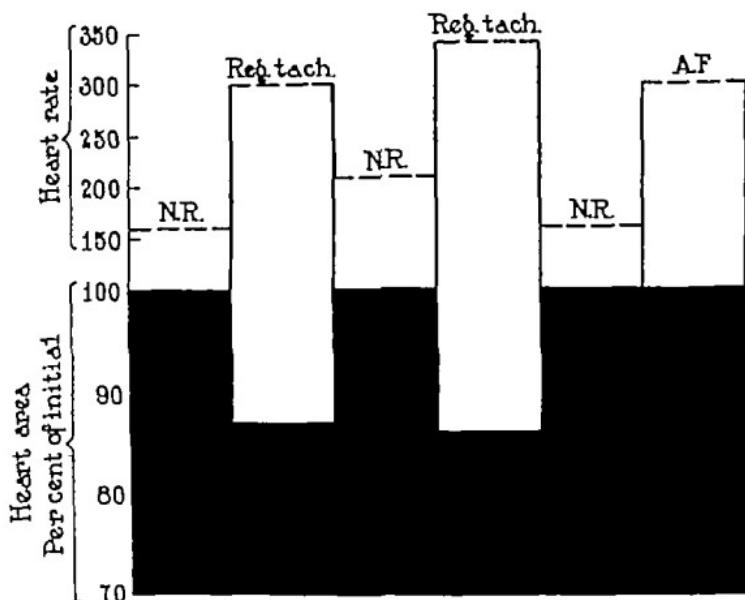


FIG. 1 In this figure is compared the effect of regular and irregular tachycardia on the size of the heart in dog 189. The size of the heart and the heart rate are seen to vary inversely during the period of regular tachycardia, while the heart area is unchanged during the period of irregular tachycardia (auricular fibrillation). N.R. = normal rhythm, Reg tach. = regular tachycardia, A.F. = auricular fibrillation

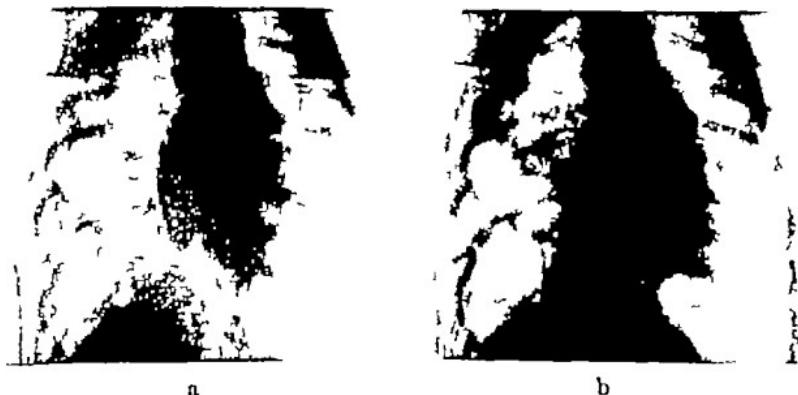


FIG. 2 Dog 189 X-ray photograph 2a was taken during the natural rate, 2b was taken after regular tachycardia had been present for 1 hour

In 7 dogs we made 8 observations on the effect of regular tachycardia on the size of the heart (table 3). It was found that the heart decreased in size in every instance, the decrease varying between 7



FIG 3 Dog 189 X-ray photograph 3a was taken during the natural rate, 3b was taken after auricular fibrillation had been present for 1 hour

TABLE 3  
*Summary of experiments*

Rhythm	Number of observations		
	Decreased	Increased	No change
Regular tachycardia	8	0	0
Auricular fibrillation	0	1	2

and 14 per cent. In 3 dogs the effect of rapid irregular tachycardia (auricular fibrillation) was compared with the effect of regular tachycardia. The size of the heart was increased in one of these observations and remained unchanged in the other two.

#### DISCUSSION

The reduction in size of the heart in dogs during regular tachycardia in distinction to that found when the rate is slow is at variance with the clinical experience that on percussion the heart appears to be dilated during paroxysms of tachycardia. This may be due either to inability to detect by percussion small differences in size of the

heart, or to the difference in reaction of normal and diseased hearts to high rates. It was furthermore surprising to find a difference in the effect of regular and irregular tachycardia on the size of the heart. Stewart, Crawford and Hastings (3, 4) have shown a difference in the response of the heart to tachycardia of the regular and irregular type in that the blood flow was always decreased during auricular fibrillation but was usually unchanged during regular tachycardia. The difference in the effect of these two mechanisms on the size of the heart is another indication of the difference in functional response of the heart to these two rhythms. What the significant correlation is, we are unable to say. There may be a connection on the one hand between the decreased blood flow in auricular fibrillation and the unchanged or increased size of the heart, or on the other between the unchanged blood flow in regular tachycardia and the decreased size of the heart. In this connection Meek (1) thought that the decrease in size of the heart which he observed during increased pulse rate was due to a failure to maintain venous pressure, that is to say, to inadequacy of the venous return to the right auricle. We have not ascertained whether this factor plays a rôle during auricular fibrillation. If the venous return remains uniform and contraction of the heart adequate, a reduction in the volume of inflow blood per beat may occur during the increased heart rate. The shadow of the heart would then decrease by the decrease in the volume of the inflow blood.

#### SUMMARY

We have observed the effect of tachycardia on the size of the x-ray shadow of the heart in normal unanesthetized dogs. We have found that

- 1 During regular tachycardia a decrease in the size of the heart occurs
- 2 During irregular tachycardia (auricular fibrillation) the size of the heart is unchanged or is increased

#### BIBLIOGRAPHY

- 1 Meek, W J Amer Jour Physiol, 1924 lxx, 385 The Effect of Changes in Pulse Rate on Diastolic Heart Size.

- 2 Hodges, P C , and Eyster, J A E Amer Jour Roent , 1924, xii, 252 Estimation of Cardiac Area in Man
- 3 Stewart, H J , Crawford, J H , and Hastings, A B Jour Clin Invest , 1926, iii, 435 The Effect of Tachycardia on the Blood Flow in Dogs I The Effect of Rapid Irregular Rhythms as Seen in Auricular Fibrillation
- 4 Stewart, H J , and Crawford, J H Jour Clin Invest , 1926, iii, 449 The Effect of Tachycardia on the Blood Flow in Dogs II The Effect of Rapid Regular Rhythm
- 5 Stewart, H J Jour Clin Invest , 1926, iii, 475 A Technique for Measuring X-ray Photographs of the Cardiac Areas of Dogs

## THE PLASMA PROTEINS IN RELATION TO BLOOD HYDRATION

### III THE PLASMA PROTEINS IN MALNUTRITION

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In previous publications from this laboratory it has been shown that patients with diabetes severe enough to cause extreme under-nutrition and evidences of protein starvation regularly have reduced plasma proteins (1) Furthermore patients with the types of nephritis which are characterized by a tendency to edema of non cardiac origin, profuse albuminuria and low plasma proteins (chronic parenchymatous nephritis of the old terminology, nephrosis or nephrotic glomerular nephritis of Volhard and Fahr) also show distinct evidences of protein starvation (2) When given diets sufficiently rich in protein these patients retain large amounts of nitrogen over long periods, without developing any increase in the non protein nitrogen concentration of the blood Since the early report further cases have been studied and previous observations have been confirmed Such an ability to store nitrogen has only been observed in patients who have previously suffered protein starvation We have, therefore, been forced to the conclusion that nephrosis is characterized by protein starvation and believe that this may play an important part in preventing the restoration of the normal plasma protein concentration

The association of low plasma proteins with evidences of protein starvation in two entirely unrelated conditions has led to the investigation of patients with malnutrition due to various causes which would presumably lead to protein starvation The results of these studies appear in the table

For the determination of plasma or serum proteins the techniques described in the previous papers of this series were employed. Blood was withdrawn with a syringe from an artery or from a vein (without the production of venous stasis) and immediately transferred to a centrifuge tube under oil, without contact with air. The oil was displaced by the blood from the tube and the latter was tightly stoppered. After centrifugation the serum or plasma was removed and analyzed for total nitrogen by the regular Kjeldahl procedure. A sample of the whole blood or the serum was also analyzed by the Folin and Wu procedure for non-protein nitrogen ( $\text{total N} - \text{non-protein N}) \times 6.25 = \text{protein}$ . For the most recent studies serum was employed, earlier plasma was used for analysis. Plasma was obtained by collecting the blood in a centrifuge tube the walls of which had been coated with enough neutral, dried potassium oxalate to make a concentration of 0.2 per cent when the tube was filled with blood.

Although the number of cases studied is comparatively limited, it includes such a variety of unrelated conditions and the results are so consistent that it seems warranted to present them and to advance the tentative hypothesis that low plasma proteins are found in patients suffering from severe malnutrition with serious depletion of the protein stores of the body and may be considered an indication of previous protein starvation in patients without obvious cardiac disease or nephritis of the hypertensive type. In the latter conditions, reduction of the proteins of the plasma which are not easily explained as the result of undernutrition may be frequently encountered.<sup>1</sup>

It must be emphasized that conditions that cause malnutrition also frequently lead to dehydration. The latter may, by producing hemoconcentration mask the plasma protein reduction. Such effects of hemoconcentration were demonstrated during diabetic acidosis (1) and are well illustrated in cases 46584 and 9002 of this study.

The term "plasma" proteins has been used throughout this discussion, although in some of the more recent studies, as indicated in the table, serum and not plasma was employed for the analyses. Serum is preferable for two reasons. It has been frequently demonstrated, most recently by Eisenman (7) from this laboratory, that the addition of oxalate to blood causes contraction of the blood cells and the passage of fluid from the cells to the plasma. Fibrinogen appears to differ from the other proteins of the plasma in both func-

<sup>1</sup> Unpublished studies

TABLE I  
*Plasma proteins in malnutrition*

Case number	Date	Plasma proteins per cent	Diagnosis and remarks	
8169	August 31, 1922	5.89	Pernicious anemia	Red blood cells 0.8 million Pitting edema of legs
	June 2, 1923	6.57	Red blood cells 0.65 million	Pitting edema of legs
33106	October 14, 1924	5.42	Pernicious anemia	Red blood cells 0.60 million Generalized subcutaneous edema, ascites and double hydrothorax
33372	May 29, 1924	3.68	Advanced pulmonary tuberculosis.	General anasarca
35686	January 19, 1925	5.87s	Tuberculous pneumonia	Marked under nutrition
35966	February 16, 1925	5.63s	Advanced disseminated pulmonary tuberculosis	Edema of legs
54894	September 2, 1926	6.04s	Abscess of lung	Marked emaciation Examination of blood when general condition was beginning to improve
29239	February 18, 1924	5.98	Esophageal carcinoma with almost complete obstruction	
	March 1, 1924	5.89	After gastrostomy when patient was improving	
26690	December 28, 1923	5.52	Carcinoma of ascending colon with almost complete obstruction	After the administration of fluids
46584	December 22, 1925	5.87s	Carcinoma of stomach with complete obstruction of pylorus	Before saline treatment, when patient was extremely dehydrated
	December 23, 1925	4.69s	After subcutaneous administration of saline	
	December 26, 1925	4.11	Three days after gastroenterostomy	
8834	April 27, 1926	5.80s	Duodenal ulcer with complete pyloric obstruction	Before treatment
9002	June 3, 1926	7.17s	Strangulated intestinal hernia with complete intestinal obstruction	Before treatment
	June 7, 1926	5.73	When patient was extremely dehydrated, and after he had received saline	
22798	September 20, 1923	4.89	Extensive burns of trunk and extremities in an old man	Fluids had been forced to the point of producing edema
46876	November 24, 1925	4.90s	Mild diabetic with arsenical dermatitis which proved fatal.	Patient was almost entirely unable to eat

TABLE 1—*Concluded*

Case number	Date	Plasma proteins*	Diagnosis and remarks	
			per cent	
34753	June 16, 1926	3 44s	Intestinal tuberculosis with severe diarrhea	
	July 1, 1926	3 56s	and extreme emaciation Pitting edema of legs and feet	
54174 22114	June 9, 1926	3 56s	Bacterial endocarditis Quite wasted	
	July 2, 1923	5 52	Adenoma of uterine cervix Cachexia Secondary anemia Red blood cells 1 10 million	
35608	January 5, 1925	5 28s	Lobar pneumonia Severe secondary anemia of unknown origin Red blood cells 2 70 million Patient had been losing weight and strength for 2 months before onset of pneumonia	
48373	September 13, 1926	3 69s	Boy, aged 6 years General peritonitis from perforated appendix had led to an anastomosis between jejunum and colon and side tracking the intermediate portions of the gut Under these conditions he had become extremely wasted and emaciated and had developed generalized subcutaneous edema and ascites	
51600	August 14, 1926	4 99s	Chronic pulmonary tuberculosis, bilateral and extensive Moderate albuminuria Patient presented slight anemia and was apparently in a fair state of nutrition	

\* For protein determinations marked s serum and not plasma was employed

tion and origin It is definitely increased, independently of the other proteins, by inflammatory or carcinomatous conditions If only total proteins are determined such fibrinogen increases may partially or entirely mask reductions of albumin and globulin

The frequency of edema in these patients is worthy of note Cachectic or nutritional edemas have been long recognized and have been ascribed by several investigators (3, 4) to protein starvation Although there is no direct relation between the occurrence of edema and the degree of plasma protein reduction, the latter, by reducing the colloidal osmotic pressure of the plasma (5, 6), must play at least

a contributory part in the production of edema just as it does in nephrosis<sup>2</sup>

#### CONCLUSIONS

Low plasma proteins have been found consistently in patients who, from a variety of causes, have developed severe malnutrition. It is suggested that reduction of the plasma proteins in individuals without obvious cardiac disease or nephritis of the types associated with hypertension and uremia, is an indication of previous protein starvation and at least a contributory cause of cachectic and starvation edemas.

#### BIBLIOGRAPHY

- 1 Peters J P , Bulger, H. A., and Eisenman, A J *Jour Clin Invest*, 1925, i, 451 The Plasma Proteins in Relation to Blood Hydration II. In *Diabetes Mellitus*
- 2 Peters, J P Bulger, H. A , Lee, Carter, and Murphy, C F *Arch Int Med* , 1926 xxxvii, 153 The Relation of Albuminuria to Protein Requirement in Nephritis
- 3 Lusk Graham *Physiol Rev* , 1921, 1, 523 The Physiological Effect of Undernurtition
- 4 Kohman E A *Amer Jour Physiol* , 1920, li, 378 The Experimental Production of Edema as Related to Protein Deficiency
- 5 Schade H , and Claussen, F *Z Klin Med* 1924, c, 363 Der onkotische Druck des Blutplasmas und die Entstehung der renal bedingten Ödems
- 6 Govaerts, P *Bull Acad roy Belgique*, 1924, (5) iv 161 Recherches cliniques sur la rôle de la pression osmotique des protéins du sang dans la pathogénie des oedèmes et de l'hypertension artérielle.
- Govaerts, P *Compt rend. soc. biol.* 1925, xcii, 441 Influence du rapport albumines-globulines sur la pression osmotique des protéins du sérum
- 7 Eisenman A J (To appear) The Effect of Potassium Oxalate on Electrolytes of Blood and Plasma

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Knack and Neumann (*D med Woch* , 1917, xlvi, 901) found low refractive indices in the sera of patients with famine edema. This is the only attempt to estimate serum proteins in this condition that we have been able to find in the literature.



# TOTAL ACID-BASE EQUILIBRIUM OF PLASMA IN HEALTH AND DISEASE

## VIII BICARBONATE AND CHLORIDE IN THE SERUM OF PATIENTS WITH HEART FAILURE

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The discovery that the "arterial" alveolar CO<sub>2</sub> (Haldane) of patients with cardiac dyspnea was low in comparison with the carbon dioxide combining capacity of the venous plasma estimated by the method of Van Slyke and Cullen (2) led Peters (1), in 1917, to conclude that the dyspnea was due to an absolute or relative carbon dioxide acidosis. This, he believed, was probably due to the fact that the respiratory mechanism for the discharge of carbon dioxide through the lungs was impaired. In order to maintain the carbon dioxide tension and the pH of the blood at or near the normal level, therefore, it was necessary to maintain the CO<sub>2</sub> tension of the effective respiratory air considerably lower. These conclusions received some support from earlier respiratory studies of Siebeck (3), Peabody (4) and others, and from subsequent investigations of Peters and Barr (5). Comparisons of the arterial CO<sub>2</sub>-tension with that of the alveolar air were made by Peters and Barr (6) in 1920 and revealed the same kind of discrepancy which Peters had earlier noted between alveolar CO<sub>2</sub> and venous plasma bicarbonate. In two patients there was also observed a definite reduction of the bicarbonate content and the pH of the arterial blood, which disappeared when compensation was established. The arterial CO<sub>2</sub> tension and pH were estimated indirectly from the CO<sub>2</sub> content of the arterial blood and the carbon dioxide absorption curve of venous blood. The results of the experiments seemed to prove conclusively that the earlier hypothesis of the authors was correct. A little later Campbell, Hunt and Poulton (7) published similar observations.

Fraser, Ross and Dreyer (8), in 1922, using Dale's colorimetric dialysis method for the determination of the pH, found that the hydrogen-ion concentration of the arterial blood was low rather than high. The low alveolar CO<sub>2</sub> they considered to be the result of over-ventilation.

At the time Peters and Barr (6) made their studies it was generally believed that arterial and venous blood were the same except for the differences of CO<sub>2</sub> and O<sub>2</sub> and that if both were exposed to the same CO<sub>2</sub> and O<sub>2</sub> tensions at the same temperature, they would be found to contain identical amounts of the two gases. Dautrebande, Meakins and Davies (9) have shown that this is not always the case and their results have been verified by the present authors (10). In its passage through the capillaries the blood may lose demonstrable amounts of water and salts. These losses are exaggerated by venous stasis and their effects are reflected in a reduction of the height of the carbon dioxide absorption curve. Meakins and his associates (9) showed that the carbon dioxide absorption curve of the venous blood of patients with decompensated heart disease was distinctly lower than that of the arterial blood, although both usually fell within the normal limits. They pointed out that the discrepancy between alveolar and arterial CO<sub>2</sub> tensions demonstrated by Peters and Barr (6) and by Campbell and Poulton (7) was not found if the arterial CO<sub>2</sub> content was compared with the arterial carbon dioxide absorption curve instead of the venous absorption curve. In their own studies Meakins and his associates detected little change in either the height of the absorption curve or the pH of the arterial blood and little or no difference between the tension of CO<sub>2</sub> of the alveolar air and that of the arterial blood.

The most striking thing about the data dealing with blood gases in arterial and in venous blood in cardiac disease which have been published by numerous observers, is their extreme variability, this is evident in studies of both oxygen and carbon dioxide. Meakins (11), in an excellent review of the subject, points out that these variations are probably referable to differences in the nature of the phenomena that characterize the condition of cardiac decompensation and shows that many of them may be explained by careful consideration of the special symptoms attending decompensation in

individual cases Like other writers on the subject, however, Meakins considers only the respiratory gases, carbon dioxide and oxygen At the present time it is well recognized that the level of bicarbonate in the blood is determined not only by the respiratory requirements of the organism, but also by the concentration of other electrolytes in the body and that, conversely, factors which influence the level of bicarbonate in the blood may also affect the concentration of other electrolytes The study of the other acids and the base of the blood might well throw some light on the nature of the disturbances which are responsible for alterations of the carbon dioxide content and the carbon dioxide capacity of the blood of patients with cardiac decompensation Accordingly, the present study was undertaken

#### METHODS OF INVESTIGATION

Blood was withdrawn usually before breakfast, either from the artery or the arm vein Arterial and venous blood are indicated in the tables by the letters "A" and "V" In taking venous blood as little stasis as possible was employed The blood thus obtained was treated in one of two ways

1 Specimens indicated in the table by the term "*cont*" were withdrawn and transferred to sampling tubes over mercury, without contact with air Blood was removed from these sampling tubes for analysis Part of the blood was transferred to centrifuge tubes which were completely filled and stoppered The plasma was removed to sampling tubes over mercury, from which samples were taken for analysis

2 Specimens indicated in the table by the term "*cap*" were brought into equilibrium with an atmosphere of a known tension of CO<sub>2</sub> in air at 38°C before analysis They were then treated like the "*cont*" specimens In most cases a tension of 40 mm. was employed In some instances samples were exposed at 30 mm and at 60 mm In these experiments, which were carried out for a different purpose, the 40 mm. point has been calculated by the straight line logarithmic formula of Peters (12)

The details of technique employed have already been described (13)

Carbon dioxide and oxygen content were determined by the methods of Van Slyke and Stadie (24) in a water jacketed constant pressure Van Slyke burette calibrated in 0.01 cc., except in a few of the most recent experiments in which CO<sub>2</sub> was determined in the new constant volume apparatus

Oxygen-capacity was estimated by a procedure in which both saturation and analysis are carried out in the Van Slyke pipette The method gives results that agree with those obtained by the technique of Van Slyke and Stadie In all except the early experiments the cell residue left after centrifugation and removal of the plasma was diluted with normal saline to the volume of the original blood and the

resulting cell mixture was used instead of the original blood for the estimation of oxygen capacity

Cell volume was determined by means of the Daland hematocrit made by the International Instrument Company for their centrifuges

For the estimation of protein 0.5 cc samples of plasma or serum were analyzed by the ordinary Kjeldahl procedure for total nitrogen. From the values thus obtained the concentration of non-protein nitrogen found by analysis of the whole blood, was subtracted. In earlier experiments the blood was prevented from clotting by the addition of sufficient dry, neutral, potassium oxalate to make a final concentration of 0.2 per cent. In later experiments the blood was defibrinated without air contact by a method previously described (13). The latter experiments are distinguished by the letter "s" after the protein value, indicating that serum and not plasma was used for analysis.

Chloride was estimated in the earlier experiments by the method of Austin and Van Slyke (14). Later the new procedure of Van Slyke (15) was used. The latter gives values usually about 3 to 4 mM lower than the older method. For most of the "cap" experiments, which are among the early studies, Austin and Van Slyke's method was used.

Inorganic phosphorus was determined by Briggs' (16) or by Benedict and Theis' (17) modification of Bell and Doisy's colorimetric method.

Total base was determined by an adaptation of Fiske's (18) method for urine analysis devised by Cullen,<sup>1</sup> except in a few of the last experiments when a modification of Stadie and Ross's (19) adaptation of Fiske's method was used. The latter is somewhat more accurate because it is not affected by the presence of phosphate.

In many of the early "cont" experiments pH was determined colorimetrically by the method of Cullen (20). For reasons that have been mentioned in an earlier paper the results thus obtained have little absolute value. In a few of the most recent experiments a gasometric technique devised by Eisenman<sup>2</sup> has been employed. This is, we believe, quite as accurate as electrometric procedures and superior to the colorimetric methods now available.

In a selected number of cases both arterial and venous blood were analyzed. In these experiments the two specimens of blood were withdrawn by two operators as nearly synchronously as possible. As soon as the first operator had entered the artery and began to withdraw blood, the second operator punctured a vein at the elbow of the opposite arm. The flow of blood from the vein was then so regulated that both samples were obtained as nearly as possible at the same time.

All values except those for oxygen capacity, cell volume and pH are calculated in terms of monovalent millimolar equivalents of acid or base. Bicarbonate is

<sup>1</sup> Personal communication

<sup>2</sup> Described without details in a previous article. Full description to be published shortly.

calculated in the "cap" experiments as *total CO<sub>2</sub>* - *dissolved CO<sub>2</sub>*. The latter is estimated by the usual solubility factors of Bohr. In the "cont" experiments when pH was determined HCO<sub>3</sub> was estimated by the following formula derived from the familiar Hasselbalch Henderson equation

$$\text{Dissolved CO}_2 = \frac{14.04 (\text{total CO}_2)}{\text{antilog} (\text{pH} - 6.1) + 1}$$

Where pH was not determined an arbitrary pH value of 7.35 was assumed and the same formula used for the calculation of dissolved CO<sub>2</sub>.

The acid value of protein was calculated by the equation of Van Slyke Wu and McLean (21)

$$\text{BP} = 0.68 \text{ P} \times (\text{pH} - 4.8)$$

Where BP represents the base-combining power of protein in millimols, P, the concentration of protein in per cent.

In 'cap' experiments pH was estimated by the Hasselbalch Henderson equation assuming a pK<sub>1</sub> value of 6.10. In "cont" experiments the observed pH value was employed when it was available. When pH was not determined an arbitrary value of 7.35 was assumed.

The acid value of phosphorus was calculated by multiplying mg of inorganic P by the factor  $\frac{1.8}{3.1}$  on the assumption that four fifths of the phosphorus exists as dibasic orthophosphate one-fifth as monobasic.

*Total acid* represents the sum of the acid values of Protein, HCO<sub>3</sub>, Cl, and PO<sub>4</sub>. The difference between this and total base has been designated as "organic acid". This fraction, of course, also includes SO<sub>4</sub>, but the concentration of the latter is usually negligibly small.

#### EXPERIMENTAL DATA AND DISCUSSION

Complete electrolyte studies were made on only a few patients. In others only certain of the constituents were determined. In all experiments chloride and bicarbonate were estimated. In table 1 are shown, for comparison, the limits of variation of the concentration of the different electrolyte constituents in the venous serum of a group of normal individuals.

Tables 2 and 3 show the variations of HCO<sub>3</sub> and Cl and their sum HCO<sub>3</sub> + Cl in normal persons or patients suffering from conditions which presumably do not affect the electrolyte picture. Table 2 includes only "V cap," table 3, "V cont" experiments. In the "cap" experiments HCO<sub>3</sub> is somewhat less variable than in the "cont"

experiments probably because variations due to differences in  $\text{CO}_2$  tension have been eliminated.  $\text{HCO}_3$  of "cap" experiments is also

TABLE 1

*Variations in the concentration of acids and base in the blood of normal individuals*

Plasma acids	Maxi mum	Mini mum	Average	Average variation		Maximum variation	
	<i>mM</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>	<i>per cent</i>	<i>mM</i>	<i>per cent</i>
Protein	13.4	10.0	11.4	±0.6	±5.5	3.4	34
Bicarbonate	30.9	21.8	25.8	±2.6	±11.9	9.1	39
Chloride	110.1	100.0	103.5	±2.8	±2.8	10.0	10
Phosphate	2.7	1.7	2.3	±0.2	±13.7	1.0	59
"Total" acid	147.5	137.8	143.3	±2.7	±1.9	9.7	7
Total base	161.3	147.2	155.7	±2.9	±1.9	14.1	10
Undetermined acid	19.8	5.8	12.1	±3.2	±55.6	14.0	241

TABLE 2

*Bicarbonate and chloride of serum from blood of normal individuals which had been brought into equilibrium with 40 mm of  $\text{CO}_2$  in air at 38°C*

Subject	Sex	HCO <sub>3</sub>	Cl	HCO <sub>3</sub> + Cl	Remarks
		<i>mM</i>	<i>mM</i>	<i>mM</i>	
JP	M	21.7	107.0	128.7	Normal
JP	M	22.4	106.5	128.9	
JP	M	23.1	103.6	126.7	
JP	M	23.0	103.0	126.0	
JP	M	22.9	103.4	126.3	
JP	M	24.4	111.2	135.6	
JP	M	23.5	109.9	133.4	
HAB	M	25.5	109.9	135.4	Normal
4729	M	22.0	108.8	130.8	Benign glycosuria
MD	F	23.1	113.0	136.1	Mild hyperthyroidism
12016	M	22.6	106.3	128.9	Neurasthenia
15004	F	25.6	109.5	135.1	Psychoneurosis
10875	F	23.3	107.2	130.5	Hysteria
10859	M	22.0	110.5	132.5	Hysteria
77914	M	25.2	110.5	135.7	Benign glycosuria
10919	M	23.5	104.0	127.5	Benign glycosuria
Average		23.4	107.8	131.6	
Maximum		25.6	113.0	136.1	
Minimum		21.7	103.0	126.0	

lower than that of "cont" experiments because the  $\text{CO}_2$  tension of venous blood is usually above 40 mm and because the carbon dioxide

capacity of the venous blood is augmented by the presence of a greater concentration of reduced hemoglobin Cl of "cap" experiments, on the other hand is higher than that of "cont" experiments and the Cl excess more than balances the CO<sub>2</sub> deficiency. The result is that the sum, HCO<sub>3</sub> + Cl, in "cap" experiments also exceeds that of "cont"

TABLE 3

*Bicarbonate and chloride content of serum from normal persons analyzed without exposure to air*

Subject	Sex	HCO <sub>3</sub>	Cl	HCO <sub>3</sub> + Cl	Remarks
		mM	mM	mM	
JP	M	29.4	99.8	129.2	Normal
		27.2	102.7	129.9	
		25.5	99.6	125.1	
		25.7	104.2	129.9	
		24.1	100.5	124.6	
		26.1	103.7	129.8	
		29.4	100.0	129.4	
		25.0	101.8	126.8	
HaB	M	27.4	98.1	125.5	Normal
		22.8	102.8	125.6	
ABD	M	26.0	102.5	128.5	Normal
		28.9	104.7	133.6	
All	M	30.9	104.2	135.1	Normal
		29.2	102.0	131.2	
Ehr	M	28.5	103.3	131.8	Normal
Hal	M	30.2	103.9	134.1	Normal
Tha	F	24.9	102.0	126.9	Normal
AJE	F	26.9	103.8	130.7	Normal
CHP	F	23.5	110.1	133.6	Mild hypothyroidism, treated
Average		26.9	102.5	129.5	
Maximum		30.9	110.1	135.1	
Minimum		22.8	98.1	124.6	

experiments. The discrepancy is only partly due to the unequal shift of Cl and HCO<sub>3</sub> in response to changes in blood reaction. Cl in all of the "cap" experiments was determined by the Austin and Van Slyke method which gives higher values than the new Van Slyke procedure which was used for a large number of the "cont" studies. As the cardiac experiments presented below were done in the same

manner and at about the same time the results are quite satisfactory for comparative purposes

TABLE 4

*Bicarbonate and chloride of serum from blood of cardiac patients which had been brought into equilibrium with 40 mm of CO<sub>2</sub> in air at 38°C*

Patient	HCO <sub>3</sub> mM	Cl mM	HCO <sub>3</sub> + Cl mM
15257	23.8	104.6	128.4
1312	22.1	104.0	126.1
15598	22.9	105.5	128.4
	24.3	110.7	135.0
10082	19.4	113.2	132.6
15534	25.2	113.8	139.0
	29.4	107.7	137.1
12862	17.9	123.7	141.8
	16.9	115.0	131.9
18292	22.7	102.3	125.0
	22.7	102.0	124.7
9426	27.2	96.6	123.8
15325	24.9	108.0	132.9
	26.8	111.2	138.0
3807	28.7	98.8	127.5
	30.5	98.8	129.3
17173	24.5	105.5	130.0
10674	24.9	106.0	130.9
	24.3	107.2	131.5
15138	24.4	103.6	128.0
12405	21.5	106.0	127.5
15751	22.6	103.0	125.6
15797	18.6	113.7	132.3
15293	22.5	106.6	129.1
3814	27.3	107.3	134.6
	24.6	106.7	131.1
	27.1	104.1	131.2
	22.8	107.3	130.1
Average	23.6	106.9	130.8
Maximum	30.5	123.9	141.8
Minimum	16.9	96.6	123.8

Tables 4 and 5, which present similar analyses of the studies on patients with cardiac disease, illustrate rather strikingly the variability of the changes found in this condition. Although the averages in

each group are quite similar to those of the comparable normals, the limits of variation of each of the two constituents and their sum is much greater among the cardiac patients. The obvious conclusion should be not that heart disease has no significant effect upon the concentration of serum  $\text{HCO}_3$  and  $\text{Cl}$ , but that the factors in cardiac

TABLE 5

*Bicarbonate and chloride content of serum from venous blood of cardiac patients, analyzed without exposure to air*

Patient	$\text{HCO}_3$	$\text{Cl}$	$\text{HCO}_3 + \text{Cl}$
18169	17.4	102.3	119.7
	22.9	99.8	122.7
	22.0	104.3	126.3
18292	24.5	106.2	130.7
	30.9	101.1	132.0
	22.7	106.5	129.2
18267	29.7	100.0	129.7
18668	33.0	89.7	122.7
18112	29.3	110.0	139.3
	30.1	101.6	131.7
	24.7	102.8	127.5
28354	20.3	109.0	129.3
	23.5	100.3	123.8
30170	24.4	104.0	128.4
35889	20.5	106.2	126.7
18667	23.3	103.5	126.8
25835	32.0	92.2	124.2
	31.8	99.3	131.1
	30.2	103.7	133.9
32567	29.9	105.6	135.5
Average	26.2	102.4	128.6
Maximum	33.0	110.0	139.3
Minimum	17.4	92.2	119.7

disease which influence the concentration of these two constituents differ in effect and probably in character. Analysis of the electrolyte data in individual cases reveals two distinct types with extreme abnormalities those with high  $\text{CO}_3$  and those with high  $\text{Cl}$ . Other types, which are somewhat less distinct present excessive deficiencies of both  $\text{CO}_3$  and  $\text{Cl}$ .

Undoubtedly retarded blood flow is one of the phenomena characteristic of heart failure. This must cause, in the terminology of Barcroft, a stagnant anoxemia, that is a condition in which the venous blood contains less than the usual amount of oxygen as compared with the arterial. By inference one would expect a proportionately great difference in the  $\text{CO}_2$  contents and the pH of the two bloods. Furthermore, the degree of venous stasis and the extent of the arterial-

TABLE 6

*Bicarbonate and chloride content of serum from arterial blood of cardiac patients analyzed without exposure to air*

Patient	HCO <sub>3</sub>	Cl	HCO <sub>3</sub> + Cl
	mM	mM	mM
18169	16.9	105.1	122.0
	23.8	100.0	123.8
	21.3	105.9	127.2
18292	21.4	108.2	129.6
	28.2	101.5	129.7
	21.8	102.4	124.2
18267	29.0	100.1	129.1
18667	31.7	90.4	122.1
18112	29.3	107.1	136.4
	28.3	101.5	129.8
	27.2	100.9	128.1
22693	25.1	96.6	121.7
26518	17.2	103.9	121.1
22084	25.9	103.6	129.5
33568	28.2	99.4	127.6
15751	23.6	106.5	130.1
Average	24.9	102.1	125.1
Maximum	31.7	108.2	136.4
Minimum	16.9	90.4	121.1

venous difference will vary in different parts of the body. Venous blood from an extremity may not then serve as a good indication of the electrolyte picture in the blood as a whole. Under the conditions of these experiments in which stasis was avoided and care was taken that the extremities were not chilled, the error entailed in using the venous blood as a criterion of the concentration of electrolytes in the arterial circulation was probably minimal. Table 6

*Differences between arterial and venous blood serum taken synchronously and analyzed without exposure to air*

shows the results of analyses of arterial blood from a group of cardiac patients. The values for  $\text{HCO}_3$  are about 2 mM lower than those of venous blood. Table 7 shows a comparison of arterial and venous blood taken synchronously from the same patients. The greatest observed difference in  $\text{HCO}_3$  was only 5 mM and this was found in a non-cardiac patient. In the others the differences are considerably less, and are no greater in cardiac patients than in normal persons. It is obvious that if blood is taken with proper precautions no great error is involved in using venous blood for the determination of electrolyte concentrations even in severe cardiac decompensation.

This does not mean that the two bloods are identical or interchangeable in any respect nor that the differences between them are in any sense predictable as regards either direction or magnitude. Meakins et al (11) found the venous blood always more concentrated than the arterial when the two differed at all significantly. Such a consistent difference does not appear in our studies which include more various material. Some experiments show that water and salt has passed from the blood to the tissues in the capillaries, others show a flow of water in the opposite direction. In every case transferred water carries Cl with it, as one would expect. Schade and Clausen (22) and Govaerts (23) believe that the interchange of water between tissues and blood is controlled largely by the relative magnitudes of hydrostatic pressure produced by the action of the heart and the vasomotor mechanism which tend to produce exudation, and the osmotic pressure of the serum proteins which tends to draw fluid from the tissues to the blood. The reaction and salt content of the blood and the tissues must also have an influence. Unless it be assumed that cardiac decompensation of all types and degrees and in all stages provokes disturbances that tend only to exudation, it should be possible to demonstrate reverse movements of fluid and salt at times. Explanations of all the combinations of water and salt exchange found in individual observations must wait until our knowledge of the forces controlling these exchanges and our methods for detecting them have advanced enormously. It has been pointed out in a previous paper (10) that such exchanges are probably part of the reaction by which the body is protected from extreme local or general disturbances of osmotic and acid-base equilibrium.

#### SUMMARY AND CONCLUSIONS

The  $\text{CO}_2$  content and the  $\text{CO}_2$  capacity of the blood in cardiac disease is more variable than in normal persons. The same holds true for the concentration of chlorides and the sum, bicarbonate + chloride. The inconsistency of these changes indicates that no

single mechanism is responsible for the electrolyte disturbances of heart disease

Two types with extreme abnormalities can be distinguished those with high CO<sub>2</sub> and those with high Cl. Other types, which are somewhat less distinct, present excesses or deficiencies of both CO<sub>2</sub> and Cl.

#### BIBLIOGRAPHY

- 1 Peters, J P Amer Jour Physiol , 1917, xlili, 113 Carbon Dioxide Acidosis, the Cause of Cardiac Dyspnea
- 2 Van Slyke, D D Jour Biol Chem , 1917, xxx, 347 Studies of Acidosis. II A Method for the Determination of Carbon Dioxide and Carbonates in Solution
- 3 Siebeck, R D Arch Klin Med , 1912, cvii, 252 Die funktionelle Bedeutung der Atemmechanik und die Lungen Ventilation bei kardialer Dyspnoe
- 4 Peabody, F W Amer Jour Med Sci , 1918, clv, 100 Cardiac Dyspnea.
- 5 Peters, J P , and Barr, D P Amer Jour Physiol , 1920, liv 307 Studies of the Respiratory Mechanism in Cardiac Dyspnea. I The Low Alveolar Carbon Dioxide of Cardiac Dyspnea.
- Peters, J P and Barr, D P Amer Jour Physiol , 1920, liv, 335 II A Note on the Effective Lung Volume in Cardiac Dyspnea
- Peters, J P , and Barr D P Amer Jour Physiol , 1920, lrv, 345 III The Effective Ventilation of Cardiac Dyspnea
- 6 Peters J P , and Barr, D P Jour Biol Chem , 1921, xlvi, 537 The Carbon Dioxide Absorption Curve and Carbon Dioxide Tension of the Blood in Cardiac Dyspnea
- 7 Campbell, J M H , Hunt, G H., and Poulton, E P Jour Path Bact., 1923, xxvi, 234 An Examination of the Blood Gases and Respiration in Disease, with Reference to the Cause of Breathlessness and Cyanosis
- 8 Fraser, F R., Ross J P , and Dreyer, N B Quart. Jour Med., 1922, xv, 195 The Reaction of the Blood in Relation to Dyspnea.
- 9 Dautrebande, L , Davies, H. W , and Meakins, J Heart, 1923, x, 133 The Influence of Circulatory Changes on the Gaseous Exchange of the Blood III An Experimental Study of Circulatory Stasis
- Meakins, J , Dautrebande, L , and Fetter, W J Heart, 1923, x, 153 IV The Blood Gases and Circulation Rate in Cases of Mitral Stenosis
- 10 Peters, J P , Bulger, H A , and Eisenman, A J Jour Biol Chem , 1926, lxvii, 165 Total Acid base Equilibrium of Plasma in Health and Disease III The Differences between Arterial and Venous Blood
- 11 Meakins, J C , and Davies, H. W Respiratory Function in Disease. Edinburgh 1925

- 12 Peters, J P *Jour Biol Chem*, 1923, lvi, 745 Studies of the Carbon Dioxide Absorption Curve of Human Blood III A Further Discussion of the Form of the Absorption Curve Plotted Logarithmically with a Convenient Type of Interpolation Chart
- 13 Peters, J P , Bulger, H A , Eisenman, A J , and Lee, Carter *Jour Biol Chem*, 1926, lvii, 141 Total Acid-base Equilibrium of Plasma in Health and Disease I The Concentration of Acids and Bases in Normal Plasma
- 14 Austin, J H , and Van Slyke, D D *Jour Biol Chem*, 1921, xlv, 461 The Determination of Chlorides in Blood Plasma
- 15 Van Slyke, D D *Jour Biol Chem*, 1923, lviii, 523 The Determination of Chlorides in Blood and Tissue
- 16 Briggs, A P *Jour Biol Chem*, 1922, lii, 13 A Modification of the Bell-Doisy Phosphate Method
- 17 Benedict, S R , and Theis, R C *Jour Biol Chem*, 1924, lx, 63 A Modification of the Molybdic Method for the Determination of Inorganic Phosphorus in Serum
- 18 Fiske, C H *Jour Biol Chem*, 1922, li, 55 A Method for the Estimation of Total Base in Urine
- 19 Stadie, W C , and Ross, E G *Jour Biol Chem*, 1925, lxv, 735 A Micro Method for the Determination of Base in Blood and Serum and Other Biological Materials
- 20 Cullen, G E *Jour Biol Chem*, 1922, li, 501 Studies of Acidosis XIX The Colorimetric Determination of the Hydrogen Ion Concentration of Blood Plasma
- 21 Van Slyke, D D , Wu, Hsien, and McLean, F C *Jour Biol Chem*, 1923, lvi, 765 Studies of Gas and Electrolyte Equilibrium in the Blood V Factors Controlling the Electrolyte and Water Distribution in the Blood
- 22 Schade, H , and Claussen, F Z *Klin Med*, 1924, c, 363 Der onkotische Druck des Blutplasmas und die Entstehung der renal bedingten Ödeme
- 23 Govaerts, Paul *Bull de l'Acad de Med de Belg*, 1924, iv, 161 Recherches cliniques sur le rôle de la pression osmotique des protéines du sang dans la pathogénie des oedèmes et de l'hypertension artérielle
- 24 Van Slyke, D D , and Stadie, W C *Jour Biol Chem*, 1921, xliv, 1 The Determination of the Gases of the Blood

## TOTAL ACID-BASE EQUILIBRIUM OF PLASMA IN HEALTH AND DISEASE

### IX HIGH SERUM BICARBONATE IN HEART FAILURE ASPHYCTIC ANOXEMIA

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In the preceding paper of this series (1) it was pointed out that the concentrations of bicarbonate and of chloride in the serum of patients with heart failure are far more variable than those of normals. From this it was inferred that the factors that provoke disturbances of these elements in heart disease are not uniform. Attention was called especially to two contrasted types of extreme abnormalities, one characterized by high bicarbonate, the other by high chloride.

The present paper will be devoted to a discussion of the production and significance of high serum bicarbonate in patients with heart failure. The technical procedures employed have been described at length in earlier articles of this series (1, 2).

Scott (3), in 1917, first called attention to the fact that the bicarbonate content and capacity of the blood and the alveolar  $\text{CO}_2$ -tension of patients with chronic emphysema were abnormally high. These observations have since then been verified by a number of investigators and similar disturbances have been found in other chronic pulmonary diseases. Essen, Kauders and Porges (4) have shown that the higher  $\text{CO}_2$  of these conditions is associated with a reduction of  $\text{Cl}^-$ .

The first three cases in table 1, although they are not cases of essential emphysema, have comparable pathologic pulmonary lesions and all present high serum carbon dioxide. The elevation of bicarbonate is in no instance so extreme as to necessitate a recession of  $\text{Cl}^-$ , although the latter is, in every case below the average normal. The

TABLE I  
Electrolyte equilibrium of serum of patients with pulmonary diseases

Patient	Oxygen capacity per cent	Cell volume per cent	Proteins per cent	$\text{HCO}_3^-$ mM	$\text{PO}_4^{2-}$ mM	$\text{Cl}^-$ mM	$\text{Na}^+$ mM	$\text{K}^+$ mM	$\text{Ca}^{2+}$ mM	$\text{Mg}^{2+}$ mM	$\text{H}_2\text{CO}_3$ mM	Organic acid 6 - 5 Base	Treatment of blood	Remarks	
														(13)	(14)
33008	—	—	—	31.4	100.5	1.9	161.1	131.9	V cont	Chronic bronchitis and emphysema	—	—	—	—	—
45918	21.3	50.5	6.15	10.7	25.7	101.8	138.2§	165.0	26.8§	127.5	V cap	Chronic bronchitis with bronchiectases	—	—	—
53439	23.9	57.8	7.08	12.3	25.9	99.0	136.9§	159.0	22.1§	124.9	V cap	Generalized tuberculous bronchopneumonia. Pleurisy with effusion	Serum pH 7.44*	—	—
24.0	58.9	8.02	13.9	26.0	99.3	139.2§	150.4	11.2§	125.3	V cap	Diffuse pulmonary tuberculosis with massive pleuritic effusion	—	—	—	—
35966	16.8	40.2	5.63	10.1	26.0	80.0	1.9	118.0§	126.4	8.4	106.0	V cont	and generalized edema. Serum pH 7.43†	—	—
33372	4.9	18.6	3.68	6.6	25.3	99.5	2.3	133.7	148.7	15.0	124.8	V cont	—	—	—

\* By new gasometric technique

† By Cullen colometric technique

§  $\text{PO}_4^{2-}$  was not determined in these cases, therefore total acid appears somewhat low and organic acid proportionately too high. The error probably does not exceed 2 mM.

sum,  $\text{HCO}_3 + \text{Cl}$  is entirely normal. All of these patients, although free from all symptoms and signs of diseases of the heart or circulatory system exhibited marked cyanosis, evidence presumably, of arterial or "anoxic" anoxemia. The latter is, in turn, an indication that the lungs are not efficiently performing their function of oxygenating the blood in the pulmonary circulation.

Because of the more rapid diffusion and greater solubility of  $\text{CO}_2$ , Van Slyke (5) and others (10) have concluded that before pulmonary impairment can result in clinically serious carbon dioxide retention the most extreme anoxemia must develop. Although these pulmonary cases cannot be said to have  $\text{CO}_2$  acidosis, they do show a high plasma  $\text{CO}_2$  and bicarbonate content, which are most easily explained as a response to a retention of carbon dioxide. This retention is attended by no reduction of pH because the retained  $\text{CO}_2$  is neutralized by base. In order that the total electrolyte concentration of the blood need not increase  $\text{Cl}^-$  is diminished, presumably excreted in the urine. The effect of the changes is quite apparent. Increase of bicarbonate permits the carbon dioxide tension of the blood and the pulmonary air to rise without disturbing the pH of the blood. Because of the higher carbon dioxide tension of the pulmonary air the individual is enabled to excrete more  $\text{CO}_2$  per unit volume of respiratory air.

It might almost be proper to speak of this group of reactions as the picture of anoxic anoxemia due to impairment of the pulmonary mechanism, a condition to which the term *asphyctic* anoxemia might well be applied. This is not meant to imply that the high  $\text{CO}_2$  is a direct response to anoxemia or one that facilitates oxygenation of the blood. Conditions which seriously interfere with the ventilation of the blood in the lungs, however, are likely to be attended by high  $\text{CO}_2$  inasmuch as they interfere with the discharge of carbon dioxide from the blood. Because the rate of diffusion of oxygen is less than that of  $\text{CO}_2$ , it is hardly conceivable that retention of  $\text{CO}_2$  can appear before a definite anoxemia has developed.

The conception that high  $\text{CO}_2$  is to be expected in asphyctic anoxemia has certain useful implications. First of all it disposes definitely of the idea that anoxemia *per se* leads to reduction of bicarbonate and alkalosis. The only type of anoxemia that has been shown to cause such changes is the anoxic anoxemia due to reduction of oxygen ten-

sion in the inspired air. There is no evidence that stagnant or anemic anoxemia have similar effects. Experimental studies have forced the conclusion that the alkalosis that results from breathing oxygen-poor air is not directly referable to the anoxemia but is due to the overventilation which this anoxemia causes. Koehler et al (6) have shown that the alkalosis can be modified to any extent by adding carbon dioxide to the inspiratory air.

Secondly, if high blood CO<sub>2</sub> is the usual reaction to asphyctic anoxemia, its absence in patients with pathologic processes that produce such a condition would indicate the presence of other factors that have a contrary effect. This is the reasoning that led the authors to propose in an earlier publication (7) that CO<sub>2</sub> retention occurred in lobar pneumonia, but was masked by the effects of temperature and changes in the other electrolytes.

That such factors may modify the electrolyte picture of asphyctic anoxemia is evidenced by the studies on the last two patients in table 1 (nos 35966 and 33372). The former, no 35966, was in the last stages of tuberculosis, with generalized bronchopneumonia, purulent bronchitis and a small pleuritic effusion. Besides this he was extremely cachectic and dehydrated and would take only enough fluid and carbohydrate to prevent starvation acidosis. He was deeply cyanotic and presented an extraordinary degree of dyspnea. The breath sounds were everywhere obscured by profuse, sticky râles. In spite of the unmistakable signs of asphyctic anoxemia serum CO<sub>2</sub> was not elevated. On the other hand, in keeping with the dehydration, serum base was extremely low, only 126 mm. This compelled an equivalent reduction of acid. Such a reduction of acid was observed, but was entirely at the expense of Cl, which is only 80 mM, bicarbonate remaining unaffected in spite of dyspnea.

The last case, no 33372, also in the terminal stages of tuberculosis, presents a more complicated picture. Besides the pulmonary lesions she presented a profound anemia and generalized edema. The latter was probably largely cachectic in origin, although the urine showed morphological changes indicative of a renal lesion. With low plasma proteins and edema, when salt has not been restricted, serum chloride is, in our experience, more frequently high than low. In this case the concentration of electrolytes, as evidenced by base, and Cl were

at the lower normal limits, while CO<sub>2</sub> was at about the usual level. With forces tending to produce an accumulation of both bicarbonate and chloride a compromise has been effected and a comparatively normal total electrolyte picture results. Such conclusions are so largely built on inference that they can be advanced only as hypotheses. Evidences of such conflicts and compromises will, however, appear with such frequency in the cardiac cases to be presented subsequently that it seems worth while to present the suggestive data from these cases for future reference and comparison.

Deficient oxygenation of the arterial blood has been observed in a certain proportion of patients with cardiac disease, especially with heart failure and evidences of pulmonary disorders such as congestion or edema of the lungs, or concomitant diseases of the lungs, such as emphysema, bronchitis or broncho pneumonia. The first 5 cases in table 2 (nos 32567, 33568, 3807, 25835 and 9426) exhibit quite clearly the high CO<sub>2</sub> of asphyctic anoxemia with low Cl. CO<sub>2</sub> + Cl is, in every case normal and total electrolytes are also normal in the four instances in which they were determined. All of these patients presented definite evidences of pathologic lesions of the lungs other than those due to heart failure.

The next four patients (nos 21460, 15325, 18112 and 18668) presented the typical electrolyte disturbance with pulmonary conditions which were entirely referable to cardiac decompensation. Both arterial and venous blood samples from the last three of these cases were examined for oxygen as well as carbon dioxide. In every instance definite arterial anoxemia was found.

Case no 18292 presents an interesting series of changes. At the time of the first observation he had distinct evidences of bronchitis and possibly bronchopneumonia as well as considerable edema. At the time of the third observation his condition was quite similar. In spite of a well marked asphyctic anoxemia CO<sub>2</sub> was at the low normal level and Cl above the average. When the second examination was made the pulmonary signs and symptoms were unrelieved, but the edema had disappeared. At this time CO<sub>2</sub> had risen above normal, and Cl had fallen, although the arterial anoxemia had diminished. If the high CO<sub>2</sub> of the second study was due to asphyctic anoxemia, something must have modified the electrolyte picture on the two other occasions, possibly edema which determined a retention

TABLE 2  
Electrolyte equilibrium of blood serum of cardiac patients with high serum  $CO_2$

21460	17	538	05	67	50	10	129	91	05	6	2	01	47	6	161	11	13	5	135	5	V cont.	Atherosclerosis hypertension heart failure. Slight hypoxia and cyanosis. Rhonchi and rales over whole chest. No edema. Serum pH 7.4†
15325	19	341	56	79		24	91	08	0						132	9	V cap	Atherosclerotic heart disease, heart failure. Rhonchi and rales over whole chest right hydrothorax. Extreme dyspnea orthopnea and cyanosis. Edema of lower extremities and trunk. Edema has disappeared Dyspnea and cyanosis continue				
22	648	06	49			26	8	111	2						138	0	V cap	A. cont. V cont.	A. cont. V cont.	Atherosclerosis with hypertension and heart disease. Heart failure. Hepatic cirrhosis. Orthopnea and hyperpnea with wheezing respiration and well marked cyanosis. Edema of extremities and trunk. Ascites Arterial O <sub>2</sub> saturation 75.4 per cent		
18112	18	138	2			29	3	107	1						136	4				Considerable subjective improvement, less dyspnea persists. Arterial O <sub>2</sub> saturation 88.2 per cent		
17	736	5				29	3	110	0						139	3				Edema confined to legs and lower trunk. Ascites		
16	536	7													129	8				Condition little changed Arterial O <sub>2</sub> saturation 77.8 per cent		
17	237	77	13												131	7						
16	235	0													128	1						
16	235	0													127	5						
18668	13	831	2												32	0						
14	632	5													32	7						
10	1	22	9												31	7						
10	5	23	6												33	0						
18292	15	433	7												90	4						
14	832	56	87												89	7						

## HIGH SERUM BICARBONATE IN HEART FAILURE

TABLE 2—Continued

Patient	Oxygen capacity		Cell volume		Protein		HCO <sub>3</sub>		PO <sub>4</sub>		Total acid 1 + 2 + 3 + 4		Base 5		Organic acid 6 - 7		HC <sub>3</sub> O <sub>4</sub> + 8		Cl <sup>-</sup> + 9		Treatment of blood		Remarks		
	vol. per cent	per cent	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM	mlM
18267	18 3 38 6		29 0	100 1			129 1		A cont																
	19 2 39 2	6 30	29 7	100 0			129 7		V cont																
18292	19 1 39 4		28 2	101 5			129 7		A cont																
cont.	19 3 39 8	7 47	30 9	101 1			132 0		V cont																
	15 3 32 9		21 8	102 4			124 2		A cont																
	15 4 33 8		22 7	106 5			129 2		V cont																

\* By new gasometric technique  
 † By Cullen colorimetric method  
 § For protein determination marked s serum and not plasma was employed

of Cl. This retention of Cl appears to have been attended in both instances by a dilution of the blood, as if the latter had shared in the edema. At least this offers the simplest explanation for the alterations of oxygen capacity, cell-volume and plasma proteins.

The last case, no 18267, raises some points of peculiar interest. The patient apparently had severe healed rheumatic valvular lesions. In spite of the absence of symptoms of decompensation and the facts that his lungs were entirely clear and his vital capacity comparatively large, he presented such striking cyanosis that a congenital heart lesion was suspected. The cyanosis was associated with an arterial oxygen saturation of only 85 per cent. The association of arterial anoxemia with normal lungs is itself presumptive evidence that, in spite of the history and physical signs the patient did have a congenital heart lesion which permitted part of the blood to pass from the right heart to the general circulation without traversing the lungs. Pearce (9), Lundsgaard and Van Slyke (9) and others have pointed out that it is impossible under these circumstances for an individual to effect complete oxygenation of the blood by increasing the pulmonary ventilation. On the other hand by hyperpnea more than the usual amount of CO<sub>2</sub> can be removed from the blood that passes through the lungs to make up for the excess CO<sub>2</sub> contributed by the unaerated blood. The carbon dioxide tension of the arterial blood of the greater circulation would, in this case, be normal, while the oxygen tension would be somewhat reduced. The possibility that compensation might be aided, as it is when the lungs are affected, by an increase of bicarbonate, is not precluded by this theory. This would, of course, afford an explanation of the high CO in this case if the patient actually had congenital heart disease.

#### SUMMARY AND CONCLUSIONS

Cardiac patients with high bicarbonate regularly present evidences of pathologic changes in the lungs which interfere with the proper ventilation of the blood, and deficient oxygenation of the arterial blood. The high bicarbonate is probably invoked in these cases as it is in pulmonary emphysema and other diseases by accumulation of carbon dioxide in the blood. It permits the tension of CO<sub>2</sub> to be maintained at a higher level than usual without reduction of pH and thus facilitates discharge of the CO<sub>2</sub> by the lungs.

If the bicarbonate rises excessively chloride is forced to recede

It is suggested that high bicarbonate and low chloride is the typical result of conditions that produce anoxic anoxemia and, at the same time, interfere with the discharge of CO<sub>2</sub> from the pulmonary circulation. To this condition the term "asphyctic" anoxemia has been applied to distinguish it from the anoxemia produced by reduction of oxygen tension in the inspired air which leads to overventilation, bicarbonate deficiency and hyperchloremia.

If the typical picture of high CO<sub>2</sub> and low Cl is not found in diseases with pulmonary lesions that lead to asphyctic anoxemia, it may be inferred that other disturbances due to unrelated factors have modified the electrolyte equilibrium. Illustrations are given of such compromises due to chloride increases or total electrolyte deficiency.

#### BIBLIOGRAPHY

- 1 Peters, J P , Bulger, H A , and Eisenman, A J *J Clin Investigation*, 1927, iii 497 Total Acid Base Equilibrium of Plasma in Health and Disease VIII Bicarbonate and Chloride in the Serum of Patients with Heart Disease
- 2 Peters, J P , Eisenman, A J , Bulger, H A , and Lee, Carter *Jour Biol Chem* , 1926, lxvii, 141 Total Acid Base Equilibrium of Plasma in Health and Disease I The Concentration of Acids and Bases in Normal Plasma
- 3 Scott, R W *Arch Int Med* , 1920, xxvi, 544 Observations on the Pathologic Physiology of Chronic Pulmonary Emphysema
- 4 Essen, H , Kauders, F and Porges, O *Wien Arch klin Med* , 1923, v, 499 Die Beziehungen der CO<sub>2</sub> Spannung der Alveolarluft zu den Chloriden des Blutserums
- 5 Van Slyke, D D *Trans Assoc Amer Phys* , 1924, xxix, 57 The Relation of Carbon Dioxide and Oxygen
- 6 Kochler, A E , Behneman, H M F , Benell, O E , and Loevenhart, A S *Amer Jour Physiol* , 1925, lxxiv, 590 The Cause of Death from Anoxemia
- 7 Peters, J P , Bulger, H A , Eisenman, A J , and Lee, Carter *Jour Biol Chem* , 1926, lxvii, 219 Total Acid Base Equilibrium of Plasma in Health and Disease V Miscellaneous Pathologic Conditions
- 8 Pearce, R G *Jour Lab Clin Med* , 1917, ii, 867 A Possible Explanation for the Cyanosis and Hyperpnea Seen in Pneumonia and Cardiac Decompensation
- 9 Lundsgaard, C , and Van Slyke, D D *Cyanosis Medicine Monographs*, Series no 2, The Williams & Wilkins Co , Baltimore
- 10 Hastings, A B , Neill, J M , Morgan, H J , and Binger, C A L *Jour Clin Invest* , 1924-1925, i, 25 Blood Reaction and Blood Gases in Pneumonia

# DIGITALIS AND DIURETICS IN HEART FAILURE WITH REGULAR RHYTHM, WITH ESPECIAL REFERENCE TO THE IMPORTANCE OF ETIOLOGIC CLASSIFI- CATION OF HEART DISEASE

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There is probably complete agreement today that digitalis affects the signs and symptoms of congestive heart failure most satisfactorily in the presence of auricular fibrillation. There is widespread agreement among American clinicians and investigators that a certain number of patients with heart failure and normal sinus rhythm respond to the administration of digitalis almost as satisfactorily as do those with auricular fibrillation, and many believe that such patients belong chiefly to the group designated as "chronic myocarditis" or "myocardial insufficiency." While this view has been expressed by many excellent observers, it remained for Luten (1) to bring forward the most convincing evidence in its support by a careful study which was the first to provide adequate control observations.

That there are several factors which influence the response of the regular heart to digitalis has long been recognized. More than a decade ago, Cohn (2) pointed out that rhythm alone was not a sufficient basis for the division of patients if the effect of digitalis was to be studied, and emphasized that the presence or absence of hypertension and the presence or absence of edema had to be considered. The value of a division into the groups which he suggested has been amply demonstrated by later work. Christian (3), Windle (4), Eggleston (5) and others have called attention to the importance of the *stage* of heart failure, stating that even when favorable results from digitalis are obtained at one time, the response to treatment becomes less satisfactory with each succeeding episode of failure.

sion of the T wave in Lead 2, or both—were observed in all except two cases, those two patients had bundle branch lesions which obscured the usual digitalis changes. Two or three days were allowed to elapse after the administration of the full dose of digitalis, it was then started in maintenance doses, usually 0.2 gram a day for five consecutive days of each week.

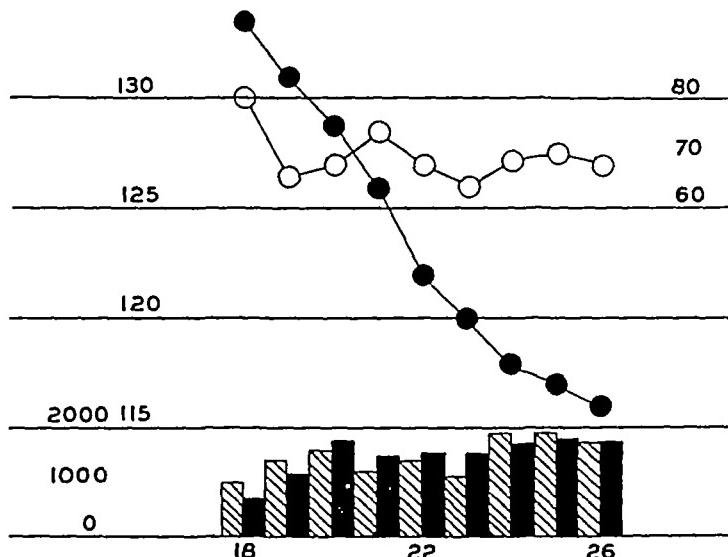


FIG 1 MALE, AGED 69 YEARS, ARTERIOSCLEROTIC HEART DISEASE WITH CONGESTIVE HEART FAILURE, RHYTHM REGULAR, BLOOD PRESSURE 132/68

No medication whatever, the only treatment consisted of rest in bed with limitation of total fluid intake to 1800 cc per day. There was slight sustained diuresis with a loss of 17 pounds in 8 days, and disappearance of the signs and symptoms of heart failure. (In this and subsequent figures, fluid intake is indicated by cross-hatched columns, urinary volume by solid columns, heart rate by circles, and weight in pounds by solid dots. Figures in the first column to the left refer to fluid intake and output, those in the second column to the weight, figures for the heart rate are on the right of the chart, and the dates are shown at the bottom.)

If satisfactory diuresis and complete disappearance of edema occurred as a result of digitalis therapy, no other drug was used. If, on the contrary, there was little or no benefit from digitalis, it was followed, after an interval of about a week, by one or more diuretics. The diuretic drugs employed were theophyllin (theocin), theobromine,

theobromine sodio-salicylate, and novasurol, the doses are indicated in the accompanying table

Attention should be directed to the fact that those individuals who derived the greatest benefit from rest alone are not included in the following analysis. Many patients were selected for study, only to be rejected later because a period of rest without medication caused the complete disappearance of the signs and symptoms of heart failure. The chart of one such patient is shown in figure 1.

So far as possible, all patients with evidence of significant renal damage have been excluded from the group. It is recognized that such evidence is often difficult of interpretation, and chief reliance has necessarily been placed upon the phenolsulphonephthalein renal function test, as customarily used, and upon the level of the blood non-protein nitrogen. These values were determined several times in the majority of patients at the time of admission to the hospital, and after the clinical condition had changed for better or for worse. Reference to table 1 will indicate that practically all members of the group had normal kidney function as judged by these tests, although a number of the elderly patients continued to show small amounts of albumin and occasional casts in the urine.

While patients were selected for study solely on the basis of rhythm and the presence of advanced congestive heart failure with considerable edema, they have been analyzed in groups based upon the etiologic type of heart disease. Three such groups are included: rheumatic, syphilitic and arteriosclerotic heart disease. A word of explanation will suffice for the first two, the third group requires brief discussion. Rheumatic heart disease was regarded as present when patients presented unequivocal signs of mitral stenosis, or mitral stenosis and aortic insufficiency, or stenosis and insufficiency of both valves. Adhesive mediastino-pericarditis was also present in some such patients, but was not encountered in the absence of valvular lesions in any subject here included. There was a history of acute rheumatic fever or acute chorea in every member of this group. The diagnosis of syphilitic heart disease was based upon signs of aortic insufficiency, with or without aneurysm, in individuals whose history included syphilitic infection, whose blood Wassermann reaction was strongly positive, and who presented no signs of valvular damage except at the

TABLE 1

Case number	Age	Sex	Duration of study	Blood pressure	Heart rate	Weight	Rest				Digitalis						
							Days	Slow ing	Symptoms improved	Loss of weight	Dose	Time	Slow ing	Symptoms improved	Loss of weight		
Arteriosclerotic heart																	
13	70	M	days 33	mm 130/76	Avg 70	pounds 112	7	0	0	3	0	1 6	2 days	0	+	10 (4 days)	+
14	50	M	32	120/90	70-80	135	1	0	0	0	0	2 2	3 days	0	+	43 (5 days)	+
15	63	M	53	132/72	80-95	155	16	0	+	14	0	2 0	30 hours	0	?	3 (2 days)	0
16	56	M	30	160-210 100	Avg 80	120	8	0	0	+5	0	2 0	1 day	0	Slight	4 (2 days)	0
17	62	M	30	120/75	80-110	150	7	0	Slight	+3	0	2 2	1 day	0	+	3 (2 days)	0
18	53	F	75	180-250 50-60	80-100	162	10	0	?	+7	0	2 4	2 days	0	0	0	0
19	58	F	50	128/80	90-106	131	14	0	+	10	0	1 2	2 days	?	+	4 (3 days) 8 (7 days) 13 (10 days)	
20	53	F	30	155/100	85-100	133	1	0	0	0	0	1 1	1 day	0	0	0	0

In this table, the total length of the period of study is given in the third column and the number of days of rest without medication in the seventh column. The heart rate given is that at the end of the rest period. The body weight is without edema if patients failed to become edema free, the weight was estimated. The figures in the column headed "X ray" indicate the total transverse diameter of the heart shadow and the transverse diameter of the thorax in the usual seven foot film. The blood non protein nitrogen is given in terms of milligrams per 100 cc., and the phenolsulphonephthalein in terms of the percent age excreted in 2 hours 10 minutes after the intramuscular injection of 1 cc. Where two figures are given in these columns, they represent the determinations at the time of admission and after clinical improvement had occurred. The + sign indicates "yes" except in the weight columns, where it means that the patient gained weight instead of losing. The period during which loss of weight occurred is given in days immediately after the figure indicating the loss. Doses are all in the metric system.

aortic orifice. Of the five such patients studied, two had aneurysm of the aortic arch, one had a diffuse dilatation of the ascending aorta, and the remaining two presented clear signs of aortitis. All five had aortic insufficiency.

—Continued

Drug	Diuretics			Edema-free Very Diameter heart Diameter thorax	Electrocardiogram	Non-protein nitrogen	Phthalolin excretion
	Dose	Loss of weight	days				
<b>disease—Continued</b>							
Theobromine so-dio-salicylate	2.6 gram 3 days	8 (3 days)	0	14 5/27 5	Normal and Vent. FB's	35	70-83
Novastrrol	2 cc.	4 (2 days)	0	18/30	Normal	30	75
Theocin	0.3 gram t.i.d. 2 days	3 (2 days)	+			46 (adm.)	68
Novastrrol	1 cc.	7 (1 day)	0	"Very large"	Normal and Vent. FB's	42	50
Theocin	0.3 gram t.i.d. 2 days	15 (2 days)	+				
Novastrrol	1 cc.	3 (1 day)	0	19 5/29	Normal	47	47
Theocin	0.3 gram t.i.d. 2 days	9 (2 days)	+				
Theobromine (2)	0.3 gram t.i.d. 2 days	0-0	0	15 5/21	Normal	30-27-21	57-85
Novastrrol (3)	1 cc.—2 cc.—2 cc.	0-0-0	0				
Theocin (3)	0.3 gram t.i.d. 2 days	23-8-8 (2 days)	+				
Theocin	0.3 gram t.i.d. 2 days	14 (2 days)	+		Intravent. Block	39	30 (adm.)
Novastrrol (2)	1 cc.—2 cc.	0-0	0	18/25 3	Left Bundle, Branch Block	41	66
Theocin (2)	0.3 gram t.i.d. 2 days	22-8 (2 days)	+				

"T.I.D." indicates that the dose mentioned was given three times a day after meals. When diuretics were given more than once, the number of occasions is indicated in parentheses after the name of the drug. Example: Case 18, a woman of 53 years with hypertension, was in the hospital for 75 days. A period of ten days rest caused no slowing of the heart rate, questionable symptomatic improvement, and a gain of 7 pounds in weight. The administration of 2.4 grams of digitalis leaf in 2 days caused no change in heart rate, symptoms, or edema. She received theobromine on two occasions and novastrrol on three without diarrhea or loss of weight. Theocin was administered three times, with pronounced diuresis and a loss of 23, 6, and 6 pounds, respectively the weight loss in each instance occurring in two days. The non-protein nitrogen was normal at three determinations, and the phthalolin excretion rose from 57 to 85 per cent after the disappearance of edema.

The term arteriosclerotic heart disease is used reluctantly, with a clear realization that it is not entirely satisfactory, but at the same time with the feeling that it is the best yet proposed of those titles which attempt to indicate etiology rather than structural changes or

functional condition. The term assumes that myocardial failure is dependent upon deficient blood flow through sclerosed coronary arteries—an assumption which is often, but not always, supported by

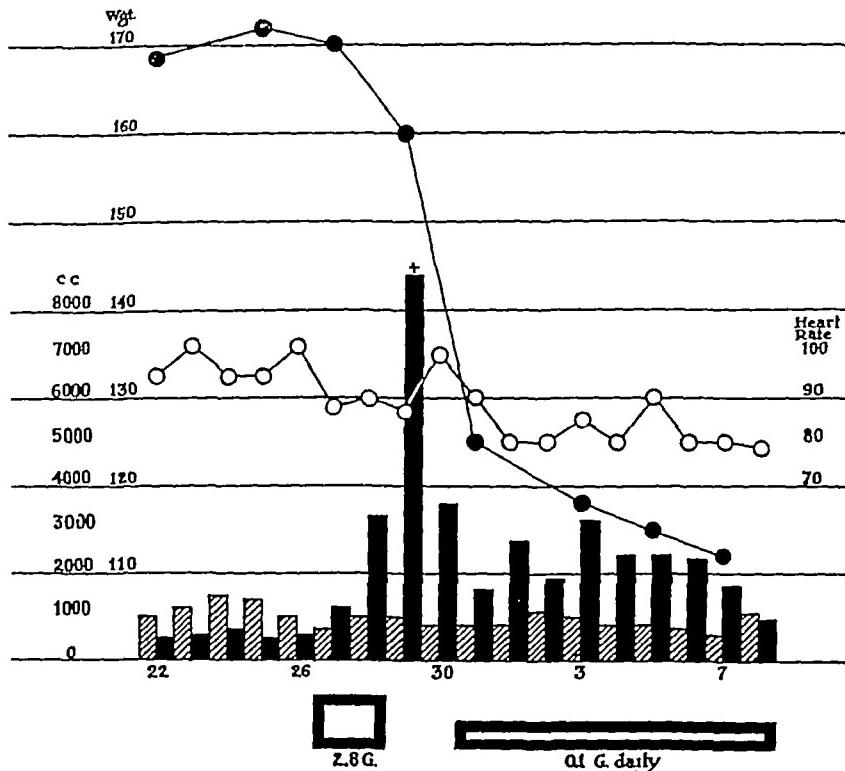


FIG 2 CASE 12 MALE, AGED 62 YEARS, ARTERIOSCLEROTIC HEART DISEASE, CONGESTIVE HEART FAILURE, REGULAR RHYTHM AND NORMAL BLOOD PRESSURE

Eight days of bed rest at home had caused no improvement, and four days of rest in the hospital resulted in an increase of 4 pounds in weight. The administration of digitalis caused intense diuresis, the urinary volume on one day rising above 9 liters, and a loss of 45 pounds in four days, with a further loss of 13 pounds in the following week. The average heart rate was slightly lowered. Digitalis is indicated by hollow rectangles at the bottom of the chart.

post-mortem examination. As used in this paper, it does not exclude—in fact, it often includes—"hypertensive heart disease." Arteriosclerosis and hypertension as etiologic factors have not been con-

sidered separately because it is so frequently impossible to determine during life which played the dominant rôle in the production of heart failure, they are often, if not always, coexistent. The term "arteriosclerotic" may therefore be read as "arteriosclerotic-hypertensive" by any who feel that the more cumbersome title is the more accurate. The diagnosis of arteriosclerotic heart disease was made in patients who showed evidences of congestive heart failure with cardiac enlarge-

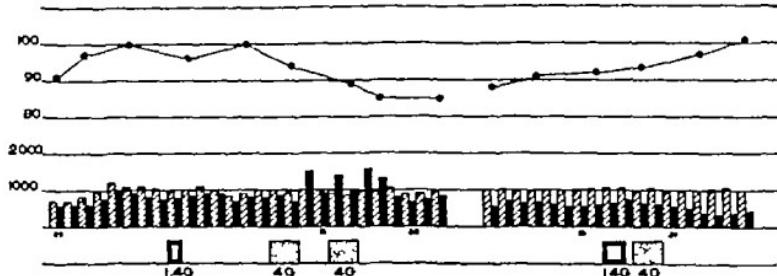


FIG 3 CASE 4 WOMAN OF 46 YEARS WITH RHEUMATIC HEART DISEASE, CONGESTIVE HEART FAILURE, REGULAR RHYTHM, EXTENSIVE EDEMA ESTIMATED WEIGHT, 70 TO 75 POUNDS

The upper figures at the left refer to the weight, the lower ones to fluid intake and output. Hollow rectangles below indicate digitalis, stippled rectangles, theocin. Maintenance doses of digitalis, 0.1 or 0.2 gram daily were given constantly after the first large dose except for one week preceding the second large dose. The gap in the chart indicates an interval of 15 days during which the weight was almost stationary. Notice that digitalis was practically without effect upon the urinary volume or weight. Theocin in twice the usual dosage caused moderate diuresis, with loss of 6 and 3 pounds. Later, however, there was no response to digitalis or theocin. At no time was this patient edema free. The heart rate was constantly between 80 and 90 per minute except for several days when there was fever.

ment, arteriosclerosis of the peripheral and retinal vessels, and no signs of valve lesions, adherent pericardium, or aortitis. No member of this group was below the age of fifty years, and none had a positive Wassermann reaction.<sup>1</sup>

<sup>1</sup> It is perhaps unnecessary to point out that the diagnosis "arteriosclerotic heart disease" corresponds closely, if not absolutely, with the terms "chronic myocarditis" and "myocardial insufficiency" which are still widely used. Of these two terms, the former is an absolute misnomer and is recognized as such even by those

The number of patients whose response to treatment has been analyzed is twenty, of which five are in the rheumatic group, five in the syphilitic, and ten in the arteriosclerotic. More than forty individuals were originally selected for study, but many had to be excluded because of incomplete data, although satisfactory conclusions could be drawn as to the nature of their response to rest and drug therapy. A few, for example, left the hospital after a stay of less than three weeks (a period which has been arbitrarily taken as the minimum for this study) although it was clear that digitalis had caused greater improvement than had rest alone. Others grew steadily worse despite rest and digitalis, but could not safely be weighed, still others could not have the renal function satisfactorily determined because of urinary incontinence or lack of cooperation. The point which deserves emphasis is that the conclusions drawn from the analysis of twenty illustrative cases are confirmed and strengthened by the study of a much larger number in which the clinical course was clear but the data were incomplete.<sup>2</sup>

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who employ it most consistently, there seems scant justification for its continued use. The latter is but a synonym of "heart failure," and is not in accord with the current belief that etiology is perhaps the most important part of a diagnosis of heart disease. The term used in this paper seems more satisfactory than either of the others in so far as it indicates the only etiology known to exist in many patients with the condition, admittedly, it does not apply accurately to all, and in view of the growing feeling that hypertension is the important cause of heart failure in such individuals, the word "hypertensive" may eventually have to be substituted for "arteriosclerotic."

<sup>2</sup>It would be incorrect, however, to permit the assumption that the total number of rheumatic and syphilitic patients was as great as the total of the arteriosclerotic group. In this clinic, the vast majority of those who have advanced congestive failure with normal rhythm fall into the last-named group, most of the rheumatics with such failure have auricular fibrillation, and congestive heart failure due to syphilis is encountered comparatively infrequently in this locality. Furthermore, relatively few of the small group of rheumatic heart disease with regular rhythm are suitable for study along the lines indicated, as most of them have received digitalis for a long period before their admission to the hospital. The same statement does not apply to the other two groups.

## RESULTS

*Rest*

There is a noticeable difference in the effect of rest upon the signs and symptoms of heart failure in the members of the different groups. The five subjects with rheumatic heart disease showed no evidence of clinical improvement or slowing of the heart rate, and three of them gained weight steadily, the other two remaining stationary. Of the five with syphilitic aortic insufficiency, three showed slight but definite improvement in respiration, and a fourth was probably a little improved, two of them lost several pounds of edema, and in two there was observed a significant reduction in the heart rate. Of the eight members of the arteriosclerotic group whose period of rest was such as to allow conclusions, slowing of the heart rate occurred in none, symptomatic improvement in three, and loss of weight in three. It is to be observed, however, that four of these patients gained weight while at rest, and the increase in weight was accompanied by other evidences of progressing heart failure. Lest these results be regarded with skepticism, as indicating less improvement from rest than has been witnessed by others, it should be reemphasized that those who derived the greatest improvement were those who lost all of their edema, and were therefore not studied.

*Digitalis*

With regard to slowing of the heart rate below the level which it reached with rest alone, it may be stated that digitalis apparently caused such slowing in none of those with rheumatic heart disease, in one with syphilitic heart disease, and in one with arteriosclerotic heart disease. This last patient, however, had been at rest for only four days, and the reduction in the average heart rate was only ten beats per minute, the result is by no means conclusive. Significant slowing occurred after the administration of digitalis in two other patients one with rheumatic, and one with arteriosclerotic heart disease, but in both of them it was due to the production of partial A-V heart block with dropped beats. It is clearly apparent that slowing of the regular heart by therapeutic doses of digitalis occurred almost never in the present group a finding which is in harmony with the conclusions of practically every other observer.

There was no symptomatic improvement in any patient with rheumatic heart disease after the administration of digitalis, three of them lost a small amount of weight, the loss being inconsiderable, and none became edema-free. Conspicuous improvement in symptoms was noted in two of the syphilitic patients, and less marked improvement in a third, four of the five had diuresis after digitalis, with complete disappearance of edema in three. The results in the arteriosclerotic group were quite different: seven of the ten had definite improvement in symptoms, and an eighth was questionably improved. In a few, the change within a period of one or two days after digitalis was little less than dramatic, and fully equal to that frequently witnessed in patients with auricular fibrillation and excessive ventricular rates. Four of them lost from 10 to 45 pounds in weight within five days or less, and were made edema-free, while four others had moderate diuresis for several days but lost only a portion of their edema. In eight of the ten members of this group, therefore, there was symptomatic improvement and loss of weight which could be ascribed to the action of digitalis.

#### *Diuretics*

All of the individuals with rheumatic heart disease received one or more of the xanthine diuretics, and one received, in addition, two doses of novasurol. Theocin caused slight diuresis in two patients, with a loss of 6 pounds in each, the other drugs employed proved useless, and no member of the group was made edema-free by any diuretic. Of the two patients with syphilitic heart disease who remained edematous after digitalis, one responded satisfactorily to novasurol and theocin, and the other grew steadily more edematous despite various diuretics. The six subjects in the arteriosclerotic group who remained edematous after digitalization were all rendered edema-free by diuretics. It happened that theocin was the drug which usually caused the final disappearance of edema, but in several instances it seemed probable that novasurol would have been just as effective. In two subjects, however (nos. 18 and 20), theocin provoked diuresis after novasurol had failed.

A word should be added concerning the several patients whose period of rest was inadequate, or whose dose of digitalis appears too

small (See table 1) The subject listed as no 2 had been in the hospital previously, it was known that rest alone caused no improvement He had been in bed at his home for thirty-three days, receiving only sedatives, and his condition at the time of entrance was such that it was deemed necessary to administer digitalis very soon in order that diuretics might be given Inasmuch as all treatment proved ineffective, the inadequacy of the rest period is of no significance Patient no 20 had received an unknown amount of digitalis before entrance, and an initial period of rest would therefore have been inconclusive She was completely digitalized and kept at rest for twenty days, with no improvement whatever, consequently the absence of a rest period is without importance The only case in which omission of the period of rest may be criticized is no 14, a man who received digitalis through error He is included in the group because the extraordinary diuresis which occurred is greater than can be explained on the basis of rest alone, his urinary volume exceeded six liters on several consecutive days, and there was a loss of 43 pounds in five days, with complete disappearance of edema No attempt is made to indicate that his improvement was due solely to digitalis, there can be little doubt that the drug was responsible for part of it The small dose of digitalis in case 7 was due to the fact that coupled rhythm invariably appeared after small amounts of the drug In cases 11 and 19, digitalis was discontinued because of headache and vomiting before the total calculated amount had been given

#### COMMENT

Conclusions cannot safely be drawn from the study of this small number of individuals, but certain tendencies seem to be sufficiently apparent to justify brief comment The feature which has seemed to us most significant is the almost absolute failure of the rheumatic heart to respond to treatment of any sort after the stage of persistent edema has been reached While that is particularly true of the heart which has maintained its normal rhythm, it is pertinent to indicate that this peculiarity of the rheumatic group may be observed even in the presence of auricular fibrillation Figure 4, which was published elsewhere to illustrate the effect of digitalis and diuretics in the arteriosclerotic groups, indicates that only 5 of 16 individuals with rheumatic heart

disease and auricular fibrillation responded satisfactorily to digitalis, while 18 of 30 similar patients in the arteriosclerotic group were freed of edema. It is generally agreed that the response of the syphilitic heart to treatment is poor, that death usually occurs within two years of the appearance of symptoms. It is of some interest, therefore, that in our experience better results may be expected in syphilitic than in rheumatic patients, if both are selected on the basis of advanced con-

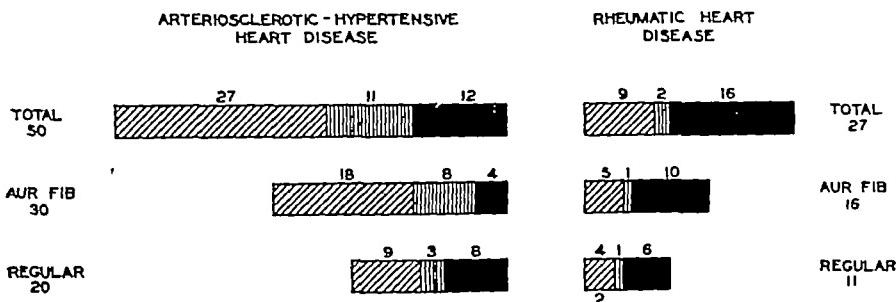


FIG 4 ILLUSTRATING THE EFFECT OF DIGITALIS AND XANTHINE DIURETICS UPON EDEMA IN PATIENTS WITH CONGESTIVE HEART FAILURE

The total number is shown in the upper column, the lower columns indicate the number with auricular fibrillation and regular rhythm. The portions of the columns in diagonal lines represent the number of patients who were freed of edema by digitalis alone, and the portions in vertical lines the number made edema-free by diuretics after digitalis had failed. The black sections indicate the number who retained part or all of their edema after digitalis and diuretics had been given repeatedly. Notice the great relative size of the black portions in the rheumatic group, even in those with auricular fibrillation. It should be noted that this chart is based solely upon the presence or absence of edema, it takes no account of symptomatic improvement. Many patients of the present series are not included in the above figures. The question mark beneath the lowest column on the right indicates that the improvement in those four individuals was probably not due solely to digitalis.

gestive heart failure with regular rhythm and considerable persistent edema. It should be added, however, that the improvement in our syphilitic patients, as in those of Scott (6), was only temporary. The far better response of the arteriosclerotic patients to treatment is so apparent as to require no emphasis. Eight of our ten patients in this group were benefited by digitalis, all were made edema-free by digitalis or diuretics.

Four of the rheumatic group died within two months, and the fifth in eleven months, after the beginning of active treatment. Four of the syphilitic patients died in one, five, six and seven months, respectively, the other is alive nine months after discharge from the hospital. Of the ten subjects in the arteriosclerotic group, five are known to be alive after periods of from four to twenty-six months, two are known to have died (one of them from pneumonia), and three cannot be traced.

It will probably be urged that the rheumatic and arteriosclerotic groups are not comparable, inasmuch as the patients in the former were near death while those in the latter were not. One purpose of this paper is to direct attention to that very fact—that the signs and symptoms usually regarded as indicating advanced heart failure represent a later stage of failure in the rheumatic than in the arteriosclerotic patient. It is quite conceivable that differences of opinion concerning the beneficial action of digitalis in heart failure may have arisen through failure to select similar patients for treatment, the basis on which selection should be made seems to us not merely the cardiac rhythm or the presence of edema, but the etiology, the rhythm and the presence of edema. If one investigator treats chiefly patients with rheumatic heart disease, and another deals almost exclusively with arteriosclerotic subjects, it is not strange that their conclusions are divergent.

Let it be clearly understood that the patients under discussion are not those with dyspnea on exertion and occasional transient edema of the lower extremities. They are individuals whose heart failure has progressed to such a point as to force the abandonment of physical activity and their confinement to bed. When this degree of failure has occurred as a result of rheumatic disease, it is seldom indeed that restoration to active life is achieved, when it has resulted from the arteriosclerotic type of heart disease, restoration to useful, though restricted, activity is often observed after rest and digitalis therapy. The syphilitic group apparently stands between the other two, although many such patients pursue the rapid downward course which characterizes the advanced failure of rheumatic heart disease.

The results in the present series of patients can be compared satisfactorily only with those obtained by Luten (1). Of his ten patients with "chronic myocarditis" (half of whom had chronic nephritis also),

nine were definitely improved by digitalis administered after a preliminary rest period, while eight of our ten with arteriosclerotic heart disease were similarly benefited. Of four cases listed as syphilitic in table 1 of his article (nos. 5, 8, 17, 18), two were improved by digitalis and two were not, results slightly less favorable than those in the present group. His case 2 is listed simply as "aortic insufficiency" but the Wassermann reaction was strongly positive and it seems probable that the valvular lesion was syphilitic rather than rheumatic, despite the age of 22 years. If that patient be grouped with the other four, it makes the results in his group and in our own almost identical. Only one of Luten's twenty patients can be classed as rheumatic heart disease (no. 7), that one was apparently improved by digitalis, but the incomplete data given indicate only slight improvement.

The observations detailed above require confirmation and extension before they can be used as a basis for definite conclusions. That is particularly true of the rheumatic and syphilitic groups, for the results in the arteriosclerotic group merely confirm the findings of many previous writers, and can scarcely be regarded as accidental. The study of this small number of patients appears to indicate that under the circumstances mentioned, digitalis and diuretic drugs yield excellent results only when administered to those with the arteriosclerotic type of heart disease. Occasionally, patients with syphilitic heart disease respond satisfactorily for a short time, but those with rheumatic heart disease do so very infrequently.

A consideration of the reason or reasons for this peculiarity of rheumatic heart disease is not here in place. Suffice it to say that in only one patient of the present group was there any evidence of infection, that one had occasional slight fever and transient leucocytosis. That a reactivation of the process in the heart may be responsible for the steady downward course of many of these individuals is suggested by a number of facts. The belief that these patients had reached a later stage of heart failure than the others has already been indicated.

#### CONCLUSIONS

The administration of digitalis in suitable large doses under properly controlled conditions to patients with advanced congestive heart failure, regular rhythm, and considerable edema, appears to cause im-

provement consistently in only one group the group here designated "arteriosclerotic heart disease," but frequently referred to as "chronic myocarditis" or "myocardial insufficiency." The drug has been found occasionally beneficial in patients with syphilitic heart disease, but almost devoid of effect in rheumatic heart disease.

The same differences have been noted with regard to the effectiveness of diuretic drugs administered after complete digitalization. Individuals with rheumatic heart disease seldom show satisfactory diuresis, those with arteriosclerotic heart disease, as a rule, respond in a highly favorable manner.

The foregoing statements are supported by the results in twenty illustrative cases analyzed in the accompanying table, but are actually based upon the study of more than twice that number.

It is suggested that differences of opinion concerning the effect of digitalis in heart failure with normal rhythm may depend in part upon differences in the patients selected for treatment. It is believed that a division of patients upon the basis of etiology of the heart disease is essential for the proper correlation of the results of different observers.

The author gratefully acknowledges the kindness of Dr E A Park and Dr Wilder Tileston, who made many helpful suggestions, and the invaluable aid of Miss Mabel Carmichael in the preparation of charts and the tabulation of data.

#### BIBLIOGRAPHY

- 1 Luten, D Arch Int Med 1924, xxxiii, 251 Clinical Studies of Digitalis  
1 Effects Produced by the Administration of Massive Dosage to Patients with Normal Mechanism
- 2 Cohn, A E Jour Amer Med Assoc., 1915, lxv, 1527 Clinical and Elec trocardiographic Studies on the Action of Digitalis.
- 3 Christian, H A Jour Amer Med Assoc., 1918, lxx, 1909 Chronic Myo carditis A Clinical Study.
- 4 Windle, J D Quart. Jour Med., 1917, x, 274 Clinical Observations on the Effect of Digitalis in Heart Disease with the Pulsus Alternans
- 5 Eggleston, C In Nelson's Loose leaf Medicine, New York, Thomas Nelson & Sons vol iv, 499 A. The Treatment of Advanced Heart Failure
- 6 Scott, R W Arch Int Med 1924 xxiv, 645 Syphilitic Aortic Insufficiency
- 7 Coombs, C T Rheumatic Heart Disease New York, William Wood & Co, 1924, p 344



## STUDIES ON RED BLOOD CELL DIAMETER<sup>1</sup>

### II IN PERNICIOUS ANEMIA, BEFORE AND DURING MARKED REMISSION, AND IN MYELOGENOUS LEUKEMIA

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#### INTRODUCTION

Since Leeuwenhoek's discovery of the human red blood corpuscles in 1673, he and many investigators have reported observations concerning their average diameter. It remained for Price-Jones (1) in 1910 to reawaken interest in such measurements by pointing out their clinical value when obtained by his simple technic and studied in a statistical manner. His (1) (2) (3) observations have been confirmed by numerous physicians (4) (5) (6) (7) and have shown that data plotted for the diameters of the red blood cells in pernicious anemia in relapse give a very wide percentage frequency curve as compared to that for normal red blood corpuscles. This indicates abnormal variation in the size of the cells. The well known fact that the average red blood cell size is distinctly greater than normal in cases of pernicious anemia with a low red blood cell count, also is emphasized by these measurements. It appears to be quite generally believed that these changes in the red blood corpuscles are present, although to a lesser degree, before the patient becomes obviously anemic and during remissions of the disease. Except for one case recorded by Price-Jones (3) with a red blood cell count of 4,400,000 per cubic millimeter, however, no data have been found in the literature concerning actual measurements of the diameters of the red blood cells when their numbers, after being distinctly few, have

<sup>1</sup> Study No I In health and pernicious anemia. Bell, J. R., Thomas, J. K., and Means, J. H. Jour Clin Invest. 1926 iii, 229

increased to above 4,200,000 per cubic millimeter Price-Jones (2) (3), Grosh and Stifel (6), and others have studied the cell diameter during remissions, but they usually have referred only to high values for hemoglobin percentage, and not to the red blood cell count. When the latter have been recorded it has been below 4,200,000 per cubic millimeter except in Price-Jones' case. These investigators found that during the remissions of their cases, although the cell size approached toward the normal, the data obtained showed that the changes in cell size typical of pernicious anemia still persisted to some degree.

We have made an investigation of the size of the red blood cells in seventeen selected cases of pernicious anemia with red blood cell counts which were low at one time but later increased, reaching in fourteen instances 4,200,000 or more per cubic millimeter. As it was thought that some cases of myelogenous leukemia might show a red blood cell picture like that of pernicious anemia in relapse, a study also was undertaken of the cell size in eleven cases of myelogenous leukemia.

#### TECHNIC AND CONTROL

The technic used to measure the size of the red blood cells was a modification of Price-Jones' method described in the first paper of the present series (7). The cells in fixed stained blood films were measured directly by means of an ocular micrometer calibrated so that measurements to every 0.625 micron could be obtained. Two hundred and fifty cells were measured for each observation recorded. From the data obtained both percentage frequency and summation frequency curves were plotted in the manner described by Bell, Thomas and Means (7) and as shown in figures 1 and 2. The median diameter is not the same as the average or mean diameter, but is the figure for the 50 percentile grade of the summation frequency curve plotted on arithmetic probability paper, and represents the center of the range of distribution of the red blood cell diameters. Thus, there are as many cells larger in diameter than the median as there are smaller in diameter. The dispersion of the red blood cell diameters is the difference between those for the 84 and the 16 percentile grades of the summation frequency curve, and indicates quantitatively the amount of anisocytosis.

Measurements were made of 2750 red blood cells in eleven preparations from six healthy individuals. The data so obtained served as a control. The average mean diameter of the normal cells was 7.55 microns, with a mean deviation in this value for the cells of the eleven smears of only  $\pm 0.16$  micron. The average median diameter was 7.50 microns, and the average dispersion was 1.17 micron. The normal extremes for these values were (a) Mean diameter from 7.25 to 7.75 microns, (b) median diameter from 7.25 to 7.75 microns, (c) dispersion from 1.00 to 1.30 micron, (d) the range of the diameters of the 2750 cells was from 5.2 to 10.0 microns. These results are comparable to those recorded by others and are in close agreement with the range for normal values obtained by Bell et al (7). These latter authors found both the normal average mean and the median diameter to be 7.70 microns, with extremes of 7.4 and 8.0 microns. The average dispersion of the normal cells they studied was 1.10 micron, with a variation of from 1.00 to 1.20 micron, and the diameters varied from 5.30 to 9.90 microns.

#### PERNICIOUS ANEMIA

Fourteen of the seventeen patients with typical pernicious anemia showed increases of red blood cell counts to 4,200,000 or more per cubic millimeter and will be particularly referred to. They improved markedly in health while taking the special diet recommended by Minot and Murphy (8) and are included in their series of 45 cases. The diet is one particularly rich in liver, contains an abundance of muscle meat, fruits, and green vegetables, and is sparing in fat. Two of the other three patients had had this diet for some weeks and their cells were measured then, when their counts were just below 4,000,000 per cubic millimeter. The last case to be recorded had a remission following no especial form of therapy.

Measurements have been made of the red blood corpuscles in eleven of the fourteen cases, both when their count was low and when it had risen to between 4,200,000 and 6,350,000 per cubic millimeter. The cells were measured in the other three cases when the red blood cell counts were over 5,000,000 per cubic millimeter but measurements could not be made when the counts were low as the preparations had been destroyed before this study was begun. However, it is

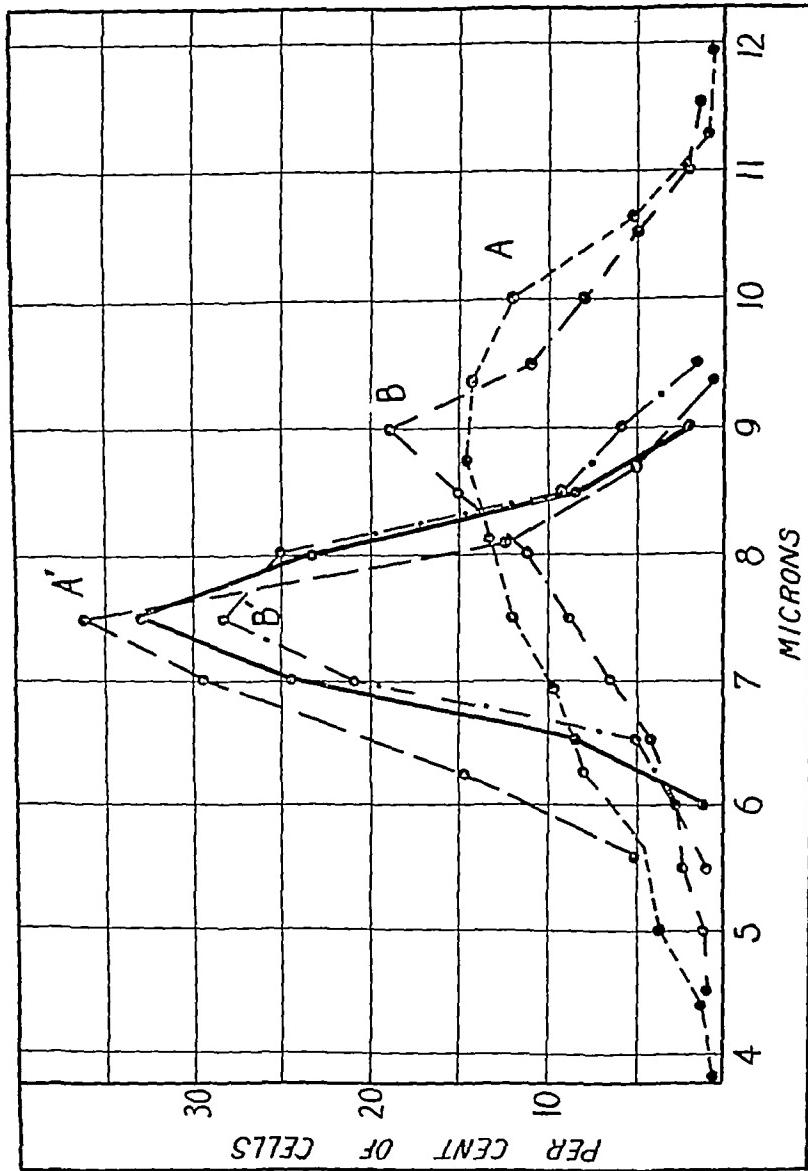


Fig 1 PERCENTAGE FREQUENCY CURVES FOR THE DIAMETERS OF THE RED BLOOD CELLS IN TWO CASES OF PERNICIOUS ANEMIA DURING RELAPSE AND MARKED REMISSION, CONTRASTED WITH NORMAL

Heavy solid line (—) normal, averaged data  
 A (—) case 7 (table 1), red blood cells 1,200,000 per cubic millimeter  
 A' (- - - - -) case 7 (table 1), red blood cells 5,020,000 per cubic millimeter  
 B (— - - - -) case 2 (table 1), red blood cells 2,400,000 per cubic millimeter  
 B' (-•-•-•-•-•) case 2 (table 1), red blood cells 4,500,000 per cubic millimeter

known that when the red blood cell counts of these three patients were low, the cells showed all the features characteristic of a relapse in pernicious anemia.

TABLE 1

*The diameters of red blood corpuscles in pernicious anemia during relapse contrasted with their diameters during marked remission*

Case	Interval between observations months	Red blood cells		Mean diameter		Median diameter		Smallest and greatest diameter		Dispersion	
		Relapse mill liters	Remission mill liters	Relapse mi crons	Remission mi crons	Relapse mi crons	Remission mi crons	Relapse microns	Remission microns	Relapse mi crons	Remission mi crons
1	9	1.1	4.2	8.70	7.57	9.10	7.71	3.7-13.0	6.3-10.0	3.80	1.24
2	20	2.4	4.4	8.43	7.62	8.71	7.69	4.0-10.0	5.5-9.5	2.54	1.34
3*	7	3.7	5.1	8.16	7.44	8.18	7.43	5.6-10.0	5.5-9.5	1.87	1.25
4{a*}	2	3.0	4.2	8.44	7.46	8.43	7.43	6.2-10.0	5.6-10.0	1.57	1.32
4{b†}	14	5.0		7.43		7.44		6.3-8.8		0.96	
5	7	2.8	4.5	8.30	7.26	8.00	7.31	3.0-13.0	5.0-8.8	2.04	1.00
6	2	2.6	4.8	8.50	7.62	8.43	7.61	4.4-11.9	5.6-10.0	2.07	1.50
7	4	1.2	5.0	8.23	7.24	8.31	7.25	3.8-12.5	5.0-9.4	3.37	1.33
8	3	1.8	5.1	7.98	7.40	7.93	7.44	5.0-11.3	5.6-9.4	2.00	1.30
9	2	2.0	5.5	7.93	6.81	7.87	6.81	5.6-11.3	5.0-8.8	2.12	1.25
10‡	2	3.2	6.4	7.46	7.23	7.46	7.25	5.0-9.4	5.6-8.8	1.70	1.06
11§	5	1.5	5.4	7.84	7.25	7.77	7.31	4.0-11.25	5.0-9.4	2.65	1.25
12	7	1.3	6.3	7.34	7.34	7.31	7.31	5.0-10.6	5.0-10.6	1.25	
13	7	2.2	6.1	7.19	7.19	7.19	7.19	5.6-10.4	5.6-10.4	1.11	
14	2	0.9	5.3	7.08	7.08	7.09	7.09	5.6-8.8	5.6-8.8	1.10	
Average	5	2.1	5.2	8.17	7.33	8.19	7.35	4.7-11.3	5.5-9.5	2.34	1.22

\* Previously the red blood cell count had been about 1,500,000 per cubic millimeter

† The lower figures (4b) are those for measurements made after patient had remained in marked remission for one year

‡ Previously macrocytosis had been marked. Liver had been eaten daily for seven weeks before first measurements were made

§ At time of relapse there were so many small cells that the values for the average diameter and median were less than is usual in pernicious anemia with low red blood cell counts. However, there is a high dispersion

¶ Measurements were not made. The cells showed a distinct macrocytosis. Microcytes were plentiful and anisocytosis marked

Table 1 summarizes the data concerning the red blood cell size of the fourteen cases. In every case during the marked remissions,

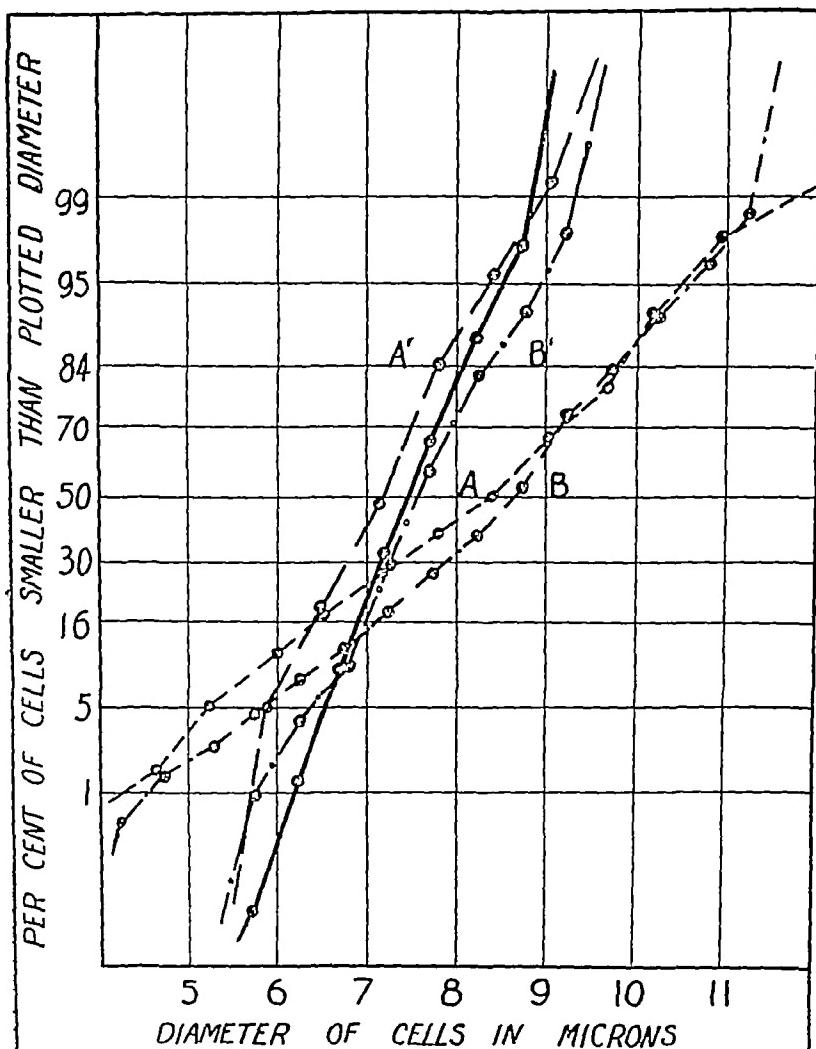


FIG 2 SUMMATION FREQUENCY CURVES PLOTTED ON ARITHMETIC PROBABILITY PAPER FOR THE DIAMETERS OF THE RED BLOOD CELLS IN THE SAME TWO CASES OF PERNICIOUS ANEMIA FOR WHICH PERCENTAGE FREQUENCY CURVES ARE GIVEN IN FIGURE 1

Solid line (—) normal, averaged data

A (— — — —) case 7 (table 1), red blood cells 1,200,000 per cubic millimeter

$A^1$  (— · — · — · — · —) case 7 (table 1), red blood cells 5,020,000 per cubic millimeter

B (— - - - -) case 2 (table 1), red blood cells 2,400,000 per cubic millimeter

$B^1$  (• - • - • - • - •) case 2 (table 1), red blood cells 4,500,000 per cubic millimeter

the values of the mean and median diameters of the red blood cells, which are abnormally high in pernicious anemia during a definite relapse, were found below the upper normal limits During remission the dispersion was found distinctly above normal (1.5 micron) only in case 6 In all but three of the other cases it was 1.3 micron or less In these three cases (2, 4a, 7) it was between 1.32 and 1.34 micron, or just above the upper limit (1.3 micron) for the normal red blood cells that we studied In one of these cases (no 4), however, the dispersion later became even less than normal (0.96 micron) In addition to this latter case, four others had a dispersion of less than 1.2 micron, the upper normal limit given by Bell, Thomas and Means (7) Thus, in spite of normal median and mean diameters there was a tendency for the dispersion to be a little above normal, but certainly five cases had a dispersion of their red blood cell diameters well below the upper normal limit

Indeed, not only did the cells in most of these fourteen cases return to normal size, but in three (cases 9, 13, and 14) their mean diameter fell distinctly below normal In four others (cases 5, 7, 10, 11), it was close to the lower normal limit Thus the curves for the diameters of the red blood cells from these cases simulated those in chronic anemia due to blood loss and other so-called secondary anemias, except that the dispersions showed a greater tendency to remain within normal limits

Figure 1 illustrates graphically by percentage frequency curves, and figure 2 by summation frequency curves, the diameters of the cells in two typical cases of pernicious anemia in relapse (cases 2 and 7 of table 1), and during remissions when there was a return of the diameters essentially or actually to normal proportions The figures in table 1 and curves of figure 1 for the diameters of the cells when their count was distinctly low, are quite like those recorded by others as typical of pernicious anemia in relapse

In the eleven cases for which measurements are recorded both before and during marked remission, it is noteworthy that in each case there was a uniform lowering of the mean and the median diameter and of the dispersion The change in the figures for the diameters was often marked, and average figures for all cases, when the red blood cell counts were low and high, as shown at the bottom

of table 1, are in striking contrast. It, thus, is evident that in marked remissions of pernicious anemia the size of the red blood corpuscles can become normal. Therefore, curves for the diameters of the red blood cells are not of diagnostic value in all stages of pernicious anemia, as concluded by Grosh and Stifel (6) and others (3) (4) (5) who have measured the red blood cells in this disease.

Data recorded in table 1 together with that given by Price-Jones (3) and others indicate that when the red blood cell count in pernicious anemia is between about 3,000,000 and 4,000,000 per cubic millimeter, although the red blood cell picture approaches normal it still retains some features typical of the disease. Two of our three patients who had received the special diet for only a few weeks and who are not included among the fourteen cases referred to above, showed, when their red blood cell count had risen to just below 4,000,000 per cubic millimeter, an abnormally large average diameter of their red blood cells, namely, 8.47 microns and 8.49 microns. The change to normal sized red blood cells, however, frequently appears to take place when the count increases somewhat above 4,000,000 per cubic millimeter, and, as it rises still higher, the average diameter of the cells may become smaller than normal.

Although the diameters of the red blood cells in cases of pernicious anemia with high red blood cell counts may not simulate those found during a relapse, this fact does not invalidate the method, in general, for diagnosis. The procedure is useful in helping to establish a diagnosis of pernicious anemia, for the patient with this disease seldom consults a physician until his red blood cell count is below 4,000,000 per cubic millimeter, and therefore at a time when the average diameter of the cells is large and when frequency curves of their diameters at least suggest pernicious anemia.

The fact that the patients with pernicious anemia who partook of the special diet, rich in liver, showed a decrease even below normal in the mean diameter of their red blood corpuscles—a finding which was associated with a decreased color index (to be discussed in a paper by Drs W P Murphy, R Fitz, and R D Monroe)—stimulates speculation. Is it possible that there is some factor in this diet which matures and produces cells at a greater rate than normal so that the size of the cells is diminished? One also wonders if patients with

pernicious anemia who have so called spontaneous remissions show cells of such small size. Probably they may do so, but we have had no opportunity to measure the cells from such a case with a distinctly high red blood cell count. The blood from one case in a spontaneous remission of over four months' duration was studied then, when the red cell count was 3,900,000 per cubic millimeter, and the hemoglobin 85 per cent. The mean diameter of these red blood cells was 7.77 microns, the median 7.75 microns, the diameters ranged from 6.25 to 10.0 microns, and the dispersion was 1.47 micron. Thus, these cells varied in size a little more than the normal, but their mean and median diameters were near the upper normal limits.

#### MYELOGENOUS LEUKEMIA

Very few measurements of the diameters of the red blood corpuscles in leukemia are recorded in the literature. Hampson and Shackle (5) state that one of three cases of "aleukemic leukemia" which they studied showed a curve for the diameter of the red blood corpuscles that was typical "for the megalocytic sort of anemia." They looked for, but never observed, this picture in chronic myelogenous leukemia.

Nine of the eleven cases of myelogenous leukemia, in which we studied the red blood cell diameter, were typical of the chronic type and showed the usual leukemic blood picture, while two were cases of subacute myelogenous leukemia, presenting an aleukemic blood picture at the time our observations were made. The former cases were treated over a period of from one to four years with roentgen-rays or radon, but no measurements of cell diameters were made in any case until the patient had remained untreated for at least four weeks and usually much longer. The two subacute cases were given no roentgen rays or radon. Measurements of the red blood cell diameters were made two to nine times in each case at intervals of weeks to months. The observations on most of the chronic cases extended over a period of two to three years. In each of the subacute cases only two observations were made, and these within a few weeks of death.

The mean diameter of the red blood cells in eight of the nine chronic cases and one of the two subacute aleukemic cases was, on nearly every observation, either definitely below normal or close to the lower

normal limit on the average about 7.10 microns and usually varying but little in each case as time passed by. The dispersion was almost always definitely increased on every examination for each case, the average figure being 1.57 micron, and the variations lying between 1.25 and 1.90 micron. In brief, the red blood cell picture was that typical of so-called secondary anemia. In two of these cases, as death approached, there was a progressive decrease in the mean diameter of the cells, in one from 7.37 to 6.94 microns, and in the other from 7.33 to 6.56 microns. The decrease in the latter case could be attributed to chronic loss of blood associated with a marked decrease in the blood platelets. There also occurred at this time many very small myeloblasts in the blood stream. In two of the other cases there developed a terminal increase in the mean diameter of the red blood cells, and this was accompanied by a rather pronounced increase in the dispersion, which rose to 1.9 micron. The mean diameter, however, never rose above the average for normal cells.

The red blood cells of one patient with chronic myelogenous leukemia were measured in preparations obtained on seven different occasions over a period of two and one-half years, and their mean diameter was always found close to 7.50 microns, which was greater than the figure observed at any time in the other chronic cases. During the following four months measurements showed that the mean diameter of this patient's red blood cells was increased distinctly above normal, reaching 8.15 microns at three months, and 8.50 microns at three weeks before death. The percentage frequency curves for the diameters of the cells from this patient, two and one-half years before, and again three weeks before death are shown in figure 3. The dispersion of the cell diameters prior to the time their mean diameter enlarged averaged 1.50 micron, and when their mean diameter increased, the degree of dispersion also rose to between 1.80 and 1.90 micron. One of the two cases of subacute myelogenous leukemia also showed, a few weeks before death, a macrocytosis of the red blood corpuscles. Their diameters varied from 5.5 to 11.25 microns with a mean diameter of 8.3 microns, and a median diameter of 8.5 microns, while the dispersion was 1.5 micron.

As the mean diameter of the red blood corpuscles increased above

normal in the chronic case, the nucleated bone marrow cells present in the peripheral blood became not only more immature in type, but also more atypical or abortive in character, although the white blood cell count remained about 70,000 per cubic millimeter. Similar cells in the subacute case formed the majority of the 5,000 white blood cells per cubic millimeter present in the peripheral blood.

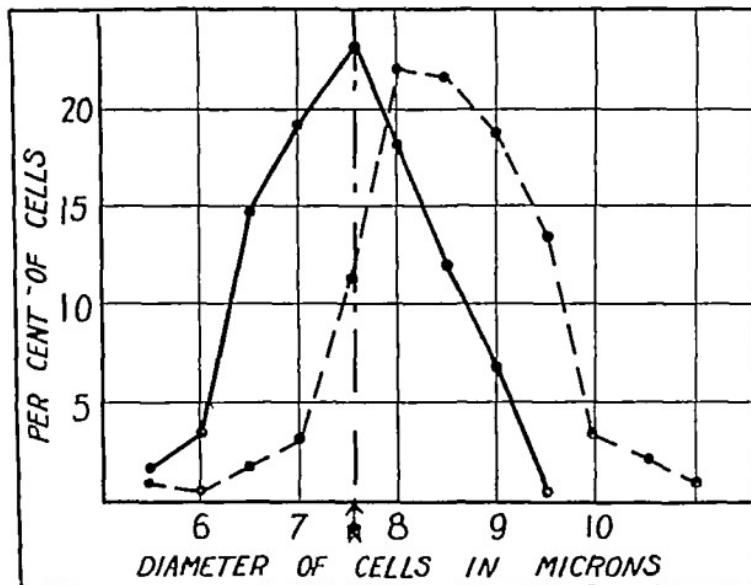


FIG. 3 CASE OF CHRONIC MYELOGENOUS LEUKEMIA THAT DEVELOPED MACROCYTOSIS OF THE RED BLOOD CELLS

Solid line (—) red blood corpuscle diameters, two and one half years before death of patient.

Dotted line (○), red blood corpuscle diameters three weeks before death of patient.

Arrow marks the locus on the curve of the averaged mean diameter for normal red blood cells.

A variety of cases with absence of free hydrochloric acid in the gastric contents, other than pernicious anemia, have shown macrocytosis of their red blood corpuscles (7). Thus one may wonder if an achlorhydria occurred in the two cases of leukemia that showed an increase in the average diameter of their red blood cells. It is known

that the patient with subacute leukemia had free hydrochloric acid in each of several specimens of gastric contents obtained after a test meal. There was no gastric analysis made in the chronic case. In both these cases of leukemia, during the latter part of the disease, the red blood cell picture simulated that of pernicious anemia in relapse, though our studies show that such a picture is not the rule in myelogenous leukemia.

#### SUMMARY

The mean and median diameter of the red blood cells in pernicious anemia may become normal (observed in 11 cases) and even less than normal (observed in 3 cases) in patients improved by a special diet rich in liver, when the red blood cell count increases to between 4,200,000 and 6,300,000 per cubic millimeter.

Under these circumstances, the dispersion of the red blood cell diameters falls and may be well below the upper normal limit, or may remain slightly above normal.

These findings occurred in cases whose red blood cell counts had previously been very low, at which time the cell diameters had all the features associated with pernicious anemia in relapse.

The measurements of the red blood cell diameters of a case of pernicious anemia in "spontaneous" remission, when the red blood cell count was 3,900,000 per cubic millimeter, showed the mean diameter to be close to the upper normal limit, but the dispersion was slightly above normal (1.47 micron). Two cases treated by the special diet for a few weeks showed a rapid increase in their red blood cells to just below 4,000,000 per cubic millimeter, at which time the size of the red blood cells showed all the features associated with pernicious anemia in relapse.

Measurements of the diameters of the red blood cells were made repeatedly in nine cases of chronic myelogenous leukemia and two of subacute aleukemic myelogenous leukemia. Usually, the red blood cells were found of a size typical of "secondary" anemia. In one case of each type the red blood cell picture simulated that of pernicious anemia in relapse, therefore a distinct macrocytosis appeared during the latter part of the disease.

## BIBLIOGRAPHY

- 1 Price Jones, C Brit. Med. Jour , 1910, ii, 1418 The Variation in the Sizes of Red Blood Cells
- 2 Price Jones, C Jour Path and Bact , 1922, xxv, 487 The Diameters of Red Cells in Pernicious Anemia and in Anemia Following Hemorrhage.
- 3 Price Jones, C Guy's Hosp Rep , 1924, lxxiv, 10 Anisocytosis with Special Reference to Pernicious Anemia
- 4 Hurst, A. F Brit. Med Jour , 1924, i, 93 An Address on Addison's (Pernicious) Anemia and Subacute Combined Degeneration of the Spinal Cord.
- 5 Hampson, A C , and Shackle, J W Guy's Hosp Rep , 1924, lxxiv, 193 Megalocytic and Non Megalocytic Anemias
- 6 Grosh L C , and Stifel, J L Arch Int Med. 1925, xxvi, 874 The Diameter of Red Blood Cells in the Differentiation of Anemias.
- 7 Bell, J R., Thomas, J K., and Means, J H Jour Cln Invest , 1926, iii, 229 Studies on Red Cell Diameter I In Health and in Pernicious Anemia.
- 8 Minot, G R , and Murphy, W P Jour Amer Med Assoc., 1926, lxxxvii, 470 Treatment of Pernicious Anemia by a Special Diet.



# THE MECHANISM OF THE ACTION OF THE HYDROGEN ION UPON THE CARDIAC RHYTHM

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It has been abundantly proved that the isolated heart will function normally for some time if it is supplied with an appropriate perfusate. It is also a striking fact that practically all of the abnormalities of rhythm encountered in the clinic are to be observed in the heart isolated from its nerve supply and beating outside the body. Inasmuch as the tissues of the heart can scarcely be thought to undergo any great organic change during the time required in isolating and starting artificial perfusion one is driven to the conclusion that in the development of these abnormal rhythms some other factor than structural damage to the cardiac muscle must play a part.

The question arises as to the similarity in fundamental origin which these abnormalities may have in common with the normal rhythm. The possibility suggests itself that the important factor may be found in some derangement of the metabolism of the cardiac tissue.

Studies of the metabolism of the myocardium have shown it to differ only quantitatively from that of other muscles. Here, as in skeletal muscle, glycogen is broken down into lactic acid which is either resynthesized to glycogen or oxidized to carbon dioxide and water. For the first process oxygen is not required. To the second it is essential. The quantitative differences between heart muscle and skeletal muscle, as shown particularly by the recent work of Katz et al (1, 2, 3) in A V Hill's laboratory are as follows:

1 The myocardium has far less capacity to "run into debt" for its oxygen.

2 Its buffering power is only one-half that of skeletal muscle. In such a tissue, alterations in the hydrogen ion concentration are rather to be expected.

The authors have for some time been engaged in studying the effects of changes in H ion concentration upon the normal rhythm of the isolated heart. The results of these studies previously reported have shown that it is possible to control the rate of the sinus or nodal rhythm by changes in the pH of the Locke perfusate between the limits of pH 7.0 and pH 7.8. With a rise in  $C_H$  the propagation of the excitatory process, as represented by the P-R interval, is retarded. When a perfusate is replaced by one slightly more alkaline, auriculo-ventricular transmission is accelerated (4). Intra-auricular conduction is similarly affected (5). The inhibitory action of the vagus is enhanced at pH 7.0 and decreased by pH 7.8 to 8.0. Sympathomimetic substances, on the other hand, are far more effective at pH 7.8 than at pH 7.0 (6). And finally, the refractory period of both auricular and ventricular muscle has been shown to vary with the reaction of the perfusate, being lengthened at pH 7.0 and shortened at pH 7.8 (7).

Such results would seem to indicate that the hydrogen ion may act to control the normal rhythmic development and transmission of the excitatory process in the heart. It remains to investigate more closely, if possible, the fundamental mechanism of this control.

#### EFFECT OF CALCIUM

It has been suggested that changes in  $C_H$  may produce their effects indirectly by effecting the ionization of the calcium. The amount of active calcium ion in such a mixture as Locke's solution is dependent chiefly upon the pH and the concentration of bicarbonate, decreasing as these factors increase. We have, therefore, undertaken to study the effects of various concentrations of calcium in Locke's solution at the same pH.

The perfusates were prepared with every precaution to insure complete solution of the calcium. NaCl, KCl, and glucose were dissolved in the usual Locke concentration. This solution was made distinctly acid by bubbling through it a mixture of oxygen and carbon dioxide, and the desired amount of calcium was then added as the chloride. Before the solutions were connected with the perfusing apparatus they were aerated with pure oxygen. The dissolved CO<sub>2</sub> was thus driven off and the reaction of the solution finally adjusted.

by the addition of NaHCO<sub>3</sub>. By this method three perfusates were made up containing respectively, 5, 10 and 15 mgm of calcium per 100 cc.

Dogs hearts were perfused through the coronary arteries by the method previously described (1) and galvanometric records taken by direct leads.

TABLE I  
*Effect of calcium concentration—5 to 15 mgm per 100 cc*

Time	Ca mgm per 100 cc	Heart rate	P R seconds	Remarks
3 41	10	65	0 17	
3 42	5			
3 45	5	65	0 16	Heart dilated, contractions weaker
3 47	5	63	0 16	
3 48	15			
3 49	15	69	0 16	
3 51	15	63	0 16	Systole augmented
3 53	15	59	0 18	
3 54	10			
3 55	10	70	0 16	
3 59	10	71	0 16	
4 00	15			
4 01	15	75	0 16	
4 02	15	75	0 16	
4 03	15	73	0 18	Contractions stronger than in normal
4 04	5			
4 05	5	73	0 16	
4 07	5	70	0 16	
4 08	10			
4 09	10	71	0 16	
4 11	10	73	0 16	

The results of a typical experiment are depicted in table 1. Throughout this study we have been unable to show that such alterations in the total calcium concentration as we have produced have consistently any effect upon the rate of development or transmission of the excitatory process. The amplitude of contraction of the heart muscle is, however, strikingly influenced by these changes. With an increase in the calcium content of the perfusate the beat becomes stronger and systole shorter. A reduction to 5 mgm per

100 cc on the other hand causes dilatation of the whole heart and an obvious reduction in the amplitude of contraction.

Andrus and Carter (8) and Daly and Clark (9) have reported an acceleration in rate and shortening of the P-R interval in the cold-blooded heart following increase in calcium. More recently Seliskar (10) working in Clark's laboratory has been unable to demonstrate that alterations in calcium content of the perfusate have any effect upon intra-auricular transmission in the turtle's heart, although such changes produce obvious differences in contraction. These results are in agreement with those of Langendorff and Hueck (11), who worked with cold and warm-blooded hearts and concluded that calcium was important only to contraction, that it had a very distinct effect in that it augmented the beat, but that it was without influence on the rate.

TABLE 2  
*Effect of phosphoric and carbonic acids on auricular rate*

pH	Auricular rate	
	Solution I	Solution II
7.8	168	168
7.6	162	168
7.4	159	166
7.2	150	162
7.0	140	155

It seems, therefore, unlikely that the effects of alterations in H ion concentration are to be explained by the resultant changes in the ionization of calcium. Indeed such positive effects as have been reported seem directly opposed to such a conclusion, since an increase in calcium has, if any, an augmentatory action whereas increase in H ion concentration is per se consistently depressant.

#### EFFECT OF $\text{H}_2\text{CO}_3$

In the course of a series of experiments upon the isolated rabbits' auricles reported by one of us (6) it was found that equal changes in pH produced quantitatively different effects upon the rhythm depending upon the acid employed. These results are shown in the

accompanying table 2 Solution I was buffered with phosphate and the changes in pH were produced with phosphoric acid. Solution II contained bicarbonate as the buffer and its reaction was controlled by the concentration of  $\text{CO}_2$  bubbled through it.

In explanation of the fact that the solution containing phosphoric acid was far more effective in depressing the rate than the perfusate

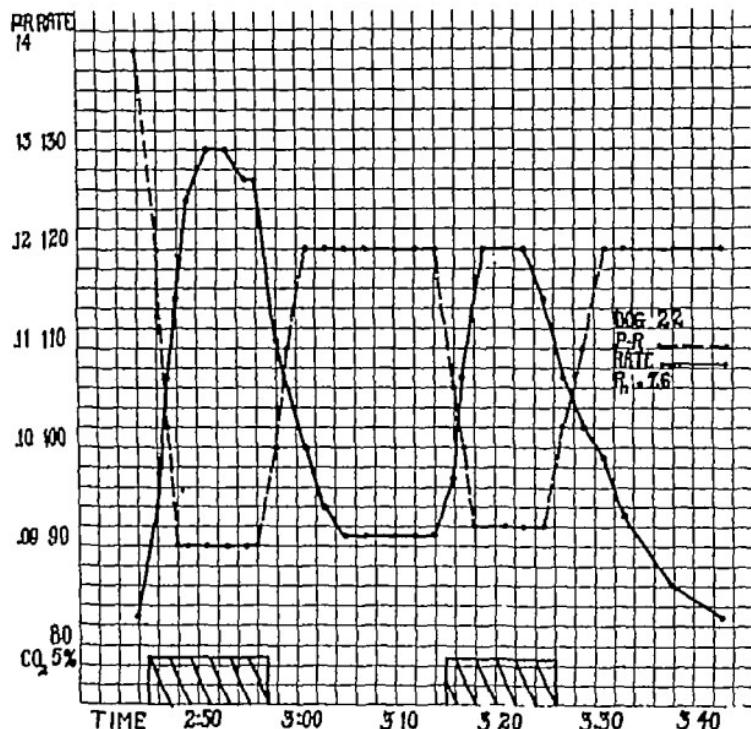


FIG. 1 THE EFFECT OF A PERFUSATE CONTAINING  $\text{CO}_2$  UPON THE EXCITATORY PROCESS IN THE DOG'S HEART

containing  $\text{CO}_2$ , it was suggested at the time that changes in the H ion concentration of the perfusing fluid when produced by carbonic acid were reflected in the C<sub>H</sub> of the cell content to a greater extent than were similar alterations brought about by phosphoric acid.

These results led us to investigate the effects upon the rhythm of different concentrations of carbonic acid at the same pH. The

hearts of dogs were perfused with two types of Locke's solution. The first was aerated with pure oxygen and its pH adjusted by the addition of  $\text{NaHCO}_3$ . The second solution was aerated with a mixture of oxygen 95 per cent and carbon dioxide 5 per cent. Samples of this solution were titrated in an atmosphere of the  $\text{CO}_2-\text{O}_2$  mixture in order to determine the amount of  $\text{NaHCO}_3$  necessary to keep the pH at the desired level. Excess of Na ion was then compensated by reducing the amount of Na added as the chloride.

When the heart is perfused with a solution containing carbonic acid the effects upon rate and transmission are very similar to those resulting upon a change from normal to a more alkaline perfusate. Thus in the experiment illustrated in figure 1, when the  $\text{H}_2\text{CO}_3$ -con-

TABLE 3  
*Effect of  $\text{CO}_2$  upon rate and conduction pH 7.2*

Time	CO <sub>2</sub> per cent	Heart rate	P R
			seconds
2 53	0	67	0 28
2 56	5	68	0 22
2 57	5	75	0 18
2 58	5	81	0 18
2 59	5	81	0 18
3 02	0	68	0 24
3 04	0	65	0 30
3 08	0	63	0 30

taining perfusate was substituted for one of the same pH (7.6) aerated with pure oxygen there was a steady rise in the rate of beat and concomitantly a shortening of the P-R interval. In table 3 are shown the results of a similar experiment carried out at pH 7.2. Here, due to the lower pH of the perfusate, the rate is slower throughout and the P-R interval longer but the same type of effect is to be noted as in the previous observation. Such results are consistently obtained, although in a certain proportion of experiments the alterations in rate come on somewhat more slowly than those resulting from a change from normal to a more alkaline perfusate, and tend to pass off more gradually upon return to the first solution.

Experiments were also carried out to determine the effects of

TABLE 4  
Influence of  $\text{CO}_2$  on transmission in auricle

Experiment	Time	pH	Auricular rate	Perfusate	Transmission mm per second
31	3 32	7 6	125	$\text{O}_2$	952
	3 36	7 6	125	$\text{O}_2$	851
	3 44	7 6	125	$\text{CO}_2$ , 5 per cent	1030
	3 47	7 6	125	$\text{CO}_2$ , 5 per cent	1090
34	3 50 $\frac{1}{2}$	7 5	190	$\text{O}_2$	859
	3 52	7 5	190	$\text{O}_2$	853
	3 55	7 5	190	$\text{CO}_2$ , 5 per cent	1080
	3 57	7 5	190	$\text{CO}_2$ , 5 per cent	1220
	4 09	7 5	190	$\text{O}_2$	955
	4 10 $\frac{1}{2}$	7 5	190	$\text{O}_2$	800
	4 13	7 5	190	$\text{CO}_2$ , 5 per cent	855
	4 14	7 5	190	$\text{CO}_2$ , 5 per cent	934
35	4 16	7 5	190	$\text{CO}_2$ , 5 per cent	955
	2 55	7 2	150	$\text{O}_2$	500
	2 56	7 2	150	$\text{O}_2$	500
	2 58	7 2	150	$\text{CO}_2$ , 5 per cent	890
	3 01	7 2	150	$\text{CO}_2$ , 5 per cent	920
	3 02	7 2	150	$\text{CO}_2$ , 5 per cent	970
	3 03 $\frac{1}{2}$	7 2	150	$\text{CO}_2$ , 5 per cent	900
	3 04 $\frac{1}{2}$	7 2	150	$\text{CO}_2$ , 5 per cent	950
	3 07	7 2	150	$\text{O}_2$	765
	3 08	7 2	150	$\text{O}_2$	700
	3 09	7 2	150	$\text{O}_2$	675
	3 10 $\frac{1}{2}$	7 2	150	$\text{O}_2$	660
	3 11 $\frac{1}{2}$	7 2	150	$\text{O}_2$	660
37	2 54	7 1	125	$\text{O}_2$	560
	2 55	7 1	125	$\text{O}_2$	530
	2 57 $\frac{1}{2}$	7 1	125	$\text{CO}_2$ , 5 per cent	975
	2 59	7 1	125	$\text{CO}_2$ , 5 per cent	800
	3 01	7 1	125	$\text{CO}_2$ , 5 per cent	825
	3 04	7 1	125	$\text{O}_2$	660
	3 05 $\frac{1}{2}$	7 1	125	$\text{O}_2$	610
	3 07	7 1	125	$\text{O}_2$	590
	3 08	7 1	125	$\text{O}_2$	550

carbonic acid upon conduction in the auricular muscle. The heart was slung in the pericardium to the thoracic walls and the right auricle was stretched out and held by means of a thread attached to

the tip of the auricular appendix Pairs of non-polarizable electrodes were placed in line in contact with the auricular muscle and each pair connected with a galvanometer string The heart was driven at a constant rate by rhythmic break shocks applied to the base of the auricle Perfusion was carried out with the solutions described above In a series of experiments the rate of transmission of the excitatory process in the auricle was consistently accelerated by a perfusate containing carbonic acid, as illustrated in table 4 Here, again is seen a gradual return to the normal rate when the oxygen-saturated perfusate replaces that containing carbonic acid

#### DISCUSSION

In earlier publications the authors have suggested that the fundamental mechanism in the control of the cardiac rhythm is dependent upon the difference in H ion concentration between cells themselves and the fluid bathing them It is as if the effect of a fall in pH in the perfusion fluid were due to a reduction in the essential gradient necessary to the spontaneous development of excitation Conversely, a rise in alkalinity of the perfusing fluid, by increasing this difference, enhances both rate and "conduction" Moreover, the fact that "conduction" is also influenced by such changes supports the view that transmission of the excitatory process depends upon the excitation of adjacent tissue by the local process at each excited point

Considerable evidence has accumulated indicating that carbonic acid or its ions exert a specific effect upon various tissues and that this property may have to do with the ability of this acid or its ions to penetrate the cell and to raise the H ion concentration of its contents This evidence is summarized in a recent paper by Smith (12) who has studied the action of various acids upon the heart muscle of the turtle with special reference to the penetration of anions The cells of the turtle's auricle, are, apparently, permeable to  $\text{CO}_2$  but impermeable to the ions of phosphoric or hydrochloric acid H ion penetrates atrial tissue only when its concentration in the tissue fluid is well above that in the cell and "in comparison with the rapid penetration of  $\text{CO}_2$ , the penetrating power of the hydrogen ion, the primary phosphate ion and lactic acid is almost negligible"

The effects of carbonic acid upon the rhythmic development and

transmission of the excitatory process may, therefore, be due to an increase in the difference in  $C_H$  within and without the cell secondary to the rise in *intra-cellular* H ion concentration. Except for the fact that these effects are less rapid in their onset and tend to persist for a certain period after the withdrawal of the  $CO_2$ ,—due possibly to conditions of diffusion,—they are identical with those obtained by changing from a normal to a more alkaline perfusate. Here again, the acceleration is, apparently, associated with an increase in the difference in H ion concentration within and without the cell.

An alternative to this explanation should be mentioned. The carbonic acid-containing solution has, obviously, by virtue of its greater bicarbonate content, a greater buffer power than the normal Locke's solution. It may, therefore, act by virtue of its *potential alkalinity*. In either case, however, the  $C_H$  of the cell itself as opposed to that of its environment would appear to be the factor controlling the rhythm.

#### SUMMARY AND CONCLUSIONS

The effects upon the rhythm of the dog's heart of changes in  $C_H$  of the perfusing fluid are not due to alterations in the ionization of calcium.

An increase in the carbonic acid content of the perfusate, brought about without change in pH, accelerates the spontaneous development and propagation of the excitatory process.

The authors conclude, therefore, that the cardiac tissue is peculiarly sensitive to alterations in hydrogen ion concentration, whether this occurs in the cell itself through products of its own metabolism or by alterations in the pH or  $CO_2$  content of the fluid bathing it, and suggest that the difference in hydrogen ion concentration within and without the cell is the factor controlling its excitation. Studies are now under way to determine the rôle of such changes in the production and control of abnormal rhythms.

#### BIBLIOGRAPHY

- 1 Katz, L N, and Long C N H Proc. Roy. Soc., Series B, 1926, xcix, 8  
Lactic Acid in Mammalian Cardiac Muscle Part I The Stimulation Maximum

- 2 Hines, H J C , Katz, L N , and Long, C N H Proc Roy Soc , Series B, 1926, xcix, 20 Lactic Acid in Mammalian Cardiac Muscle Part II The Rigor Mortis Maximum and the Normal Glycogen Content
- 3 Katz, L N , Kerridge, P I , and Long, C N H Proc Roy Soc , Series B, 1926, xcix, 26 Part III Changes in Hydrogen Ion Concentration
- 4 Andrus, E C , and Carter, E P Heart, 1924, xi, 97 The Development and Propagation of the Excitatory Process in the Perfused Heart
- 5 Drury, A M , and Andrus, E C Heart, 1924, vi, 389 The Influence of Hydrogen-Ion Concentration upon Conduction in the Auricle of the Perfused Mammalian Heart
- 6 Andrus, E C Jour Physiol , 1925, lix, 361 The Effect of Certain Changes in the Perfusate upon the Isolated Auricles of the Rabbit
- 7 Carter, E P , and Dieuaide, F R Johns Hopkins Hosp Bull , 1926, xxxix, 99 The Influence of Changes in Hydrogen Ion Concentration upon the Refractory Period of the Perfused Mammalian Heart
- 8 Andrus, E C , and Carter, E P Amer Jour Physiol , 1922, lxi, 227 The Effect upon the Cold-blooded Heart of Changes in the Ionic Content of the Perfusate
- 9 Daly, I de B , and Clark, A J Jour Physiol , 1921, liv, 367 The Action of Ions upon the Frog's Heart
- 10 Sehskar, A Jour Physiol , 1926, lxi, 172 The Action of Ions upon Intra-auricular Conduction in the Tortoise
- 11 Langendorff, O , and Hueck, W Arch f d ges Physiol , 1903, xcvi, 473 Die Wirkung des Calcium auf das Herz
- 12 Smith, H W Amer Jour Physiol , 1926, lxxvi, 411 The Action of Acids on Turtle Heart Muscle with Reference to the Penetration of Anions

## EPINEPHRIN REACTION IN OBESITY

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That a departure from the usual in metabolism, either qualitative or quantitative must exist in obesity seems self evident. In many instances, of course, the direction of this departure is plain and lies in a food intake obviously excessive. In other cases the nature of the metabolic fault is obscure.

Aside from excessive food intake, the fundamental cause of obesity conceivably may lie in a depressed rate of basal metabolism or in a lowered metabolic response to any of the calorigenic stimuli that affect the human body. Studies of the basal metabolic rate in obesity have been made in this laboratory and several reports published (1). No consistent variation from normal has been found. Confirmatory evidence has been reported by Boothby and Sandiford from the Mayo Clinic (2). More recently Strouse and his co workers have made studies in the specific dynamic action of various food substances and find that the specific dynamic action of protein is lowered in the so-called constitutional obesity (3). A similar finding has been reported by Plaut (4), but denied by Lauter (5), who states that "in the varied types of obesity, the specific dynamic action of protein often gave very high values and on the whole they were not lowered." He ascribes the origin of obesity to the food intake which is more than sufficient for the energy needs and a deposition of fat then results.

It is well known that thin persons are often nervous and emotional, and fat ones seldom so. For this reason, in the further search for a metabolic fault, it seemed advisable to study the physiological metabolic response to emotion in the obese. Since we could provide no standardizable psychic stimulus, and since in view of Cannon's work (6) it is reasonable to assume that any psychic stimulus affects

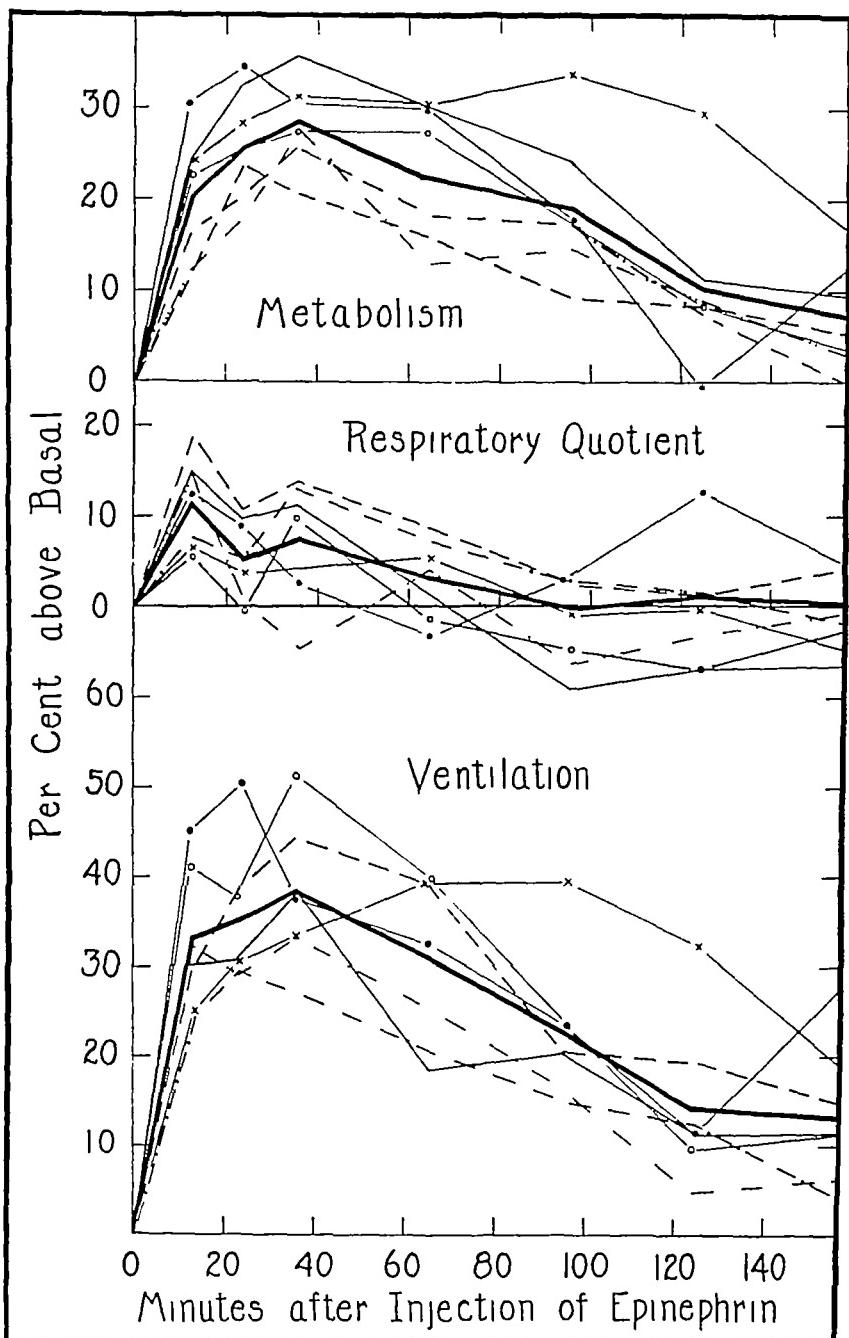


FIG 1 METABOLIC RATE, RESPIRATORY QUOTIENT AND VENTILATION AFTER EPINEPHRIN INJECTION IN SEVEN OBESE SUBJECTS

The heavy lines are the composite curves for the group, the thin lines those for the individual subjects. Each type of line denotes a particular subject throughout.

Results are all expressed as per cent above or below the basal readings, that is to say all curves start at 0, which is the basal before the drug was given, so as to make the curves comparable.

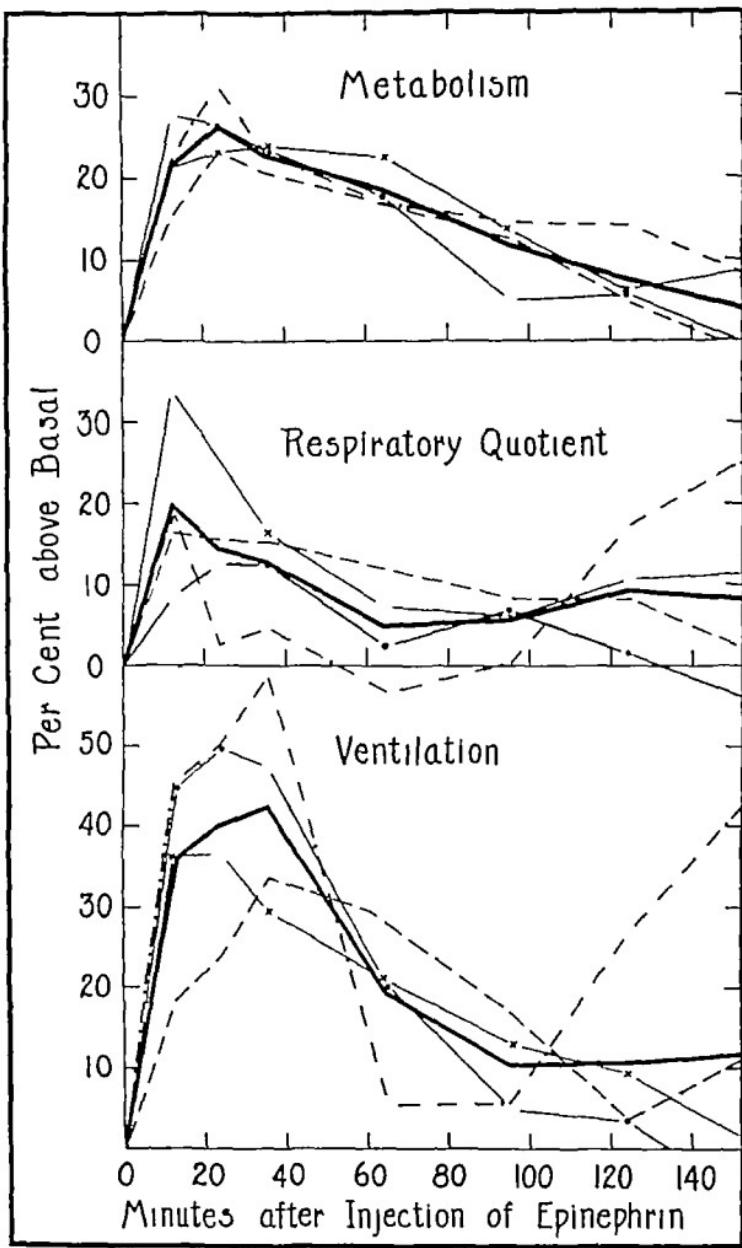


FIG. 2 METABOLIC RATE, RESPIRATORY QUOTIENT AND VENTILATION AFTER EPINEPHRIN IN THE NORMAL SUBJECTS

metabolism through its effect on epinephrin secretion, we decided to attack our problem by observing the metabolic response to the direct

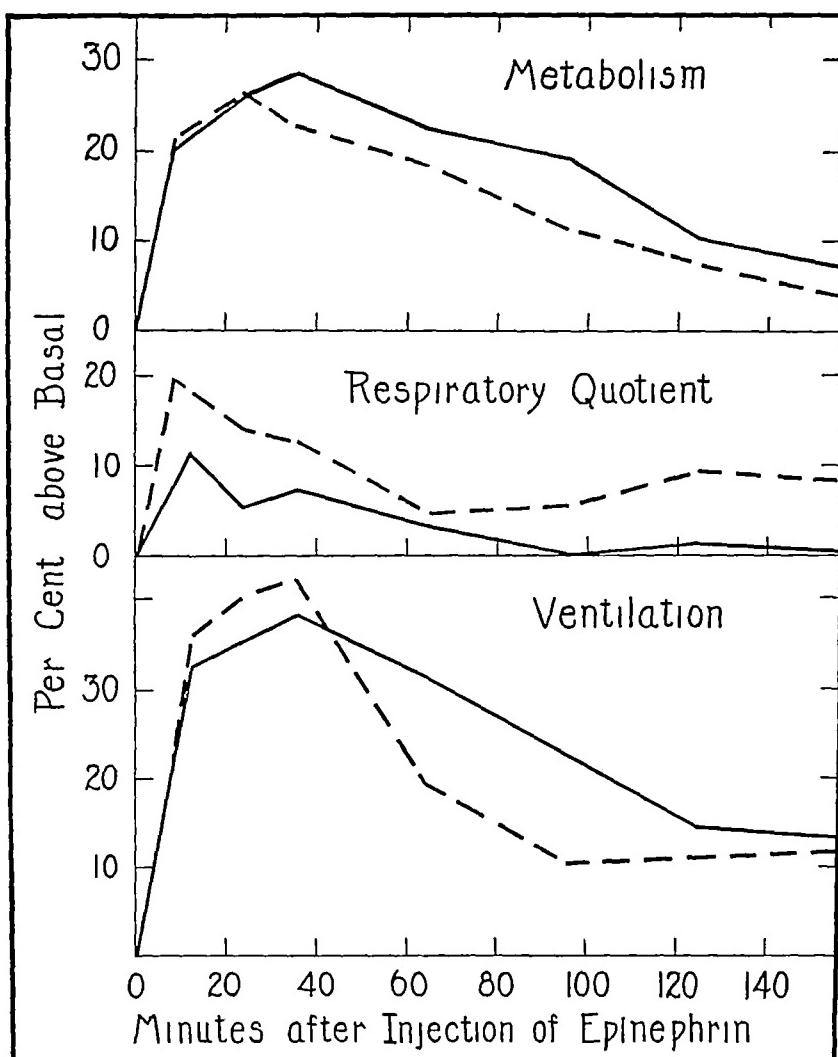


FIG 3 COMPOSITE CURVES FROM FIGURES 1 AND 2 SHOWN TOGETHER  
Heavy lines show obese and dotted lines normal subject curves

injection of epinephrin Tompkins, Sturgis and Wearn (7) have reported the effects of epinephrin injection on basal metabolism in

cases with "irritable heart" and Sandiford (8) has studied its effect on heat production, blood pressure and pulse rate in man. The

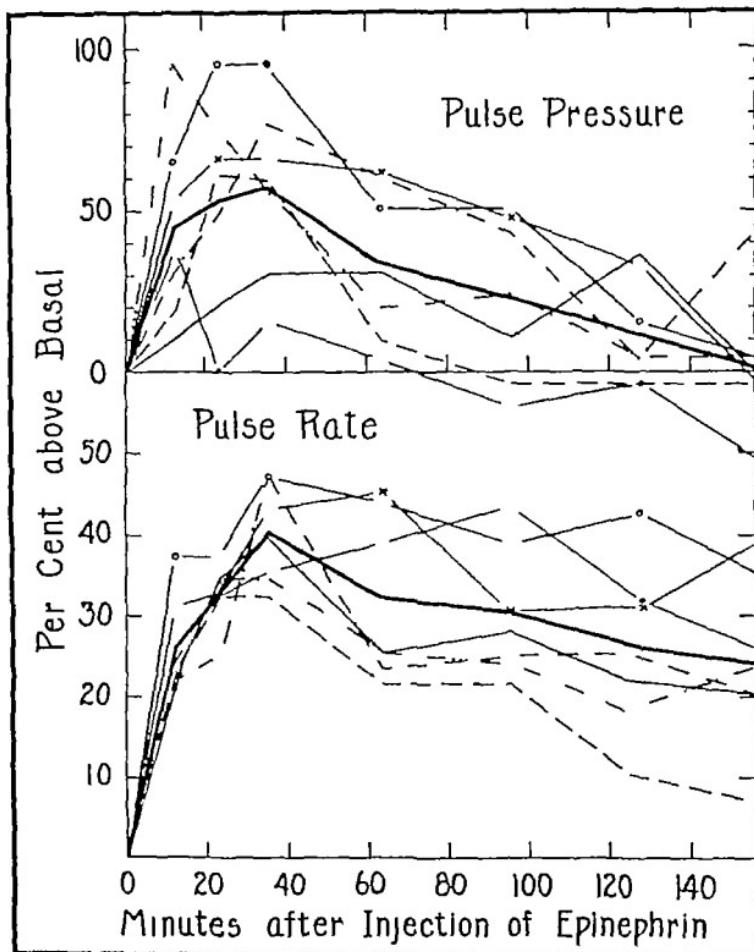


FIG. 4 PULSE PRESSURE AND PULSE RATE FOR THE EXPERIMENTS SHOWN IN FIGURE 1, OBESE SERIES

present report gives the result of such a study in a series of obese patients. In addition to the metabolic rate itself, we have likewise

observed the changes in respiratory quotient, pulmonary ventilation, pulse rate and blood pressure. The obese persons studied were free

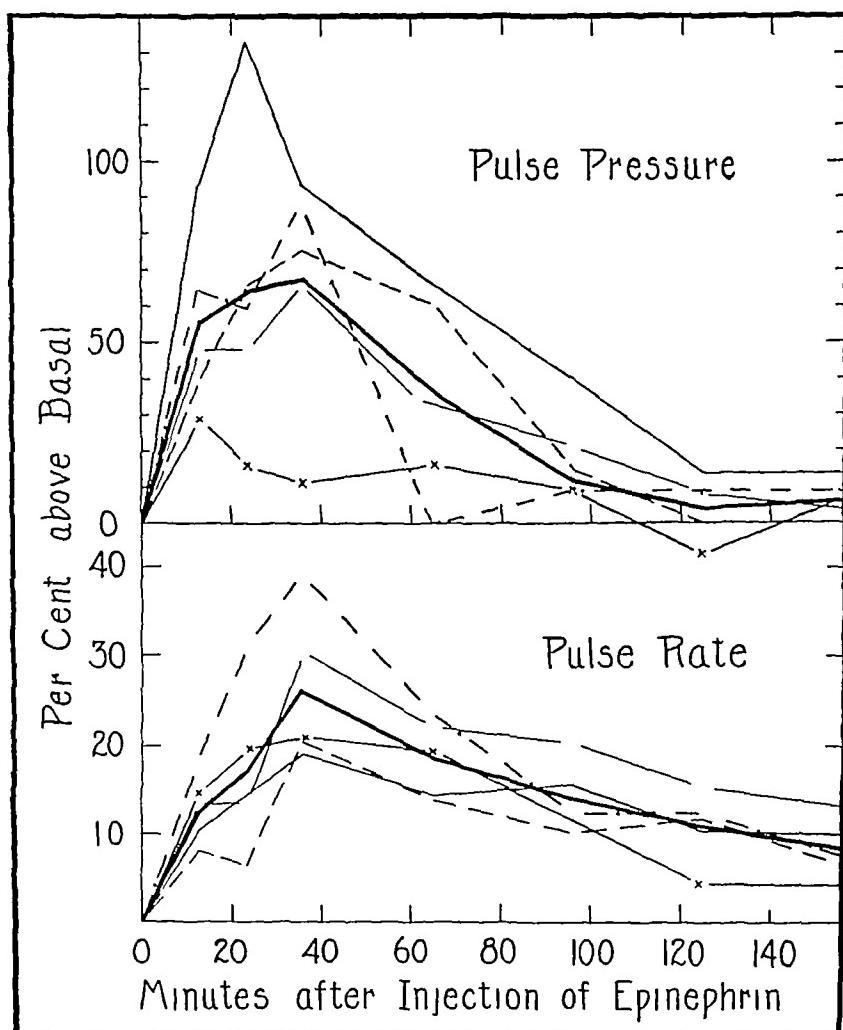


FIG. 5 PULSE PRESSURE AND PULSE RATE FOR THE EXPERIMENTS SHOWN IN FIGURE 2, NORMAL SERIES

from apparent endocrine disturbance and belonged to the type that may be called simple obesity

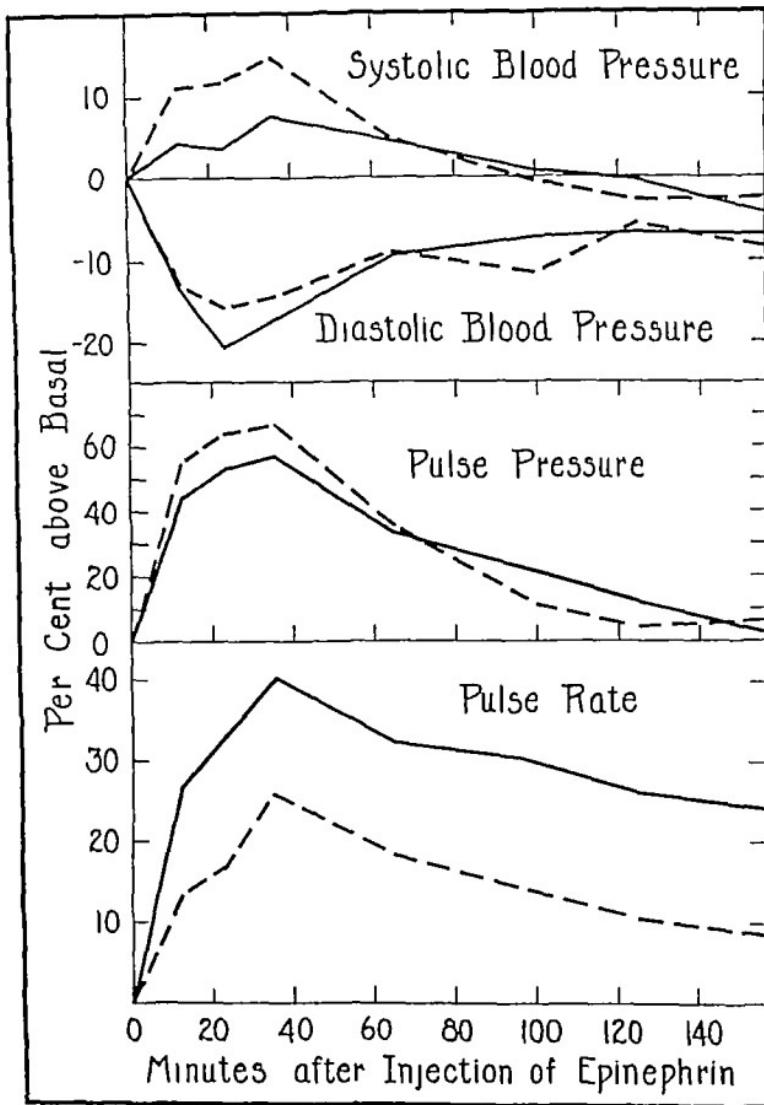


FIG 6 COMPOSITE CURVES OF SYSTOLIC AND DIASTOLIC BLOOD PRESSURES,  
PULSE PRESSURE AND PULSE RATE FOR BOTH SERIES

Heavy line = obese, dotted line = normal

## METHODS

The patients were all studied in the post absorptive condition, twelve hours or more after the last meal. On arriving at the laboratory the patient was given a preliminary rest-period of 30 minutes before any determinations were made. Then two metabolism readings were made and the lower of the two was taken as the basal. Blood pressure and pulse rate readings were taken at the same time. Epinephrin was then injected into the arm of each individual and readings started at 10, 20, 30, 60, 90, 120 and 150 minutes after injection. The patients remained in the resting condition throughout the experiment.

The metabolic studies were all made on a Tissot gasometer, from which duplicate samples of expired air were withdrawn and analyzed for oxygen and carbon dioxide by the usual Haldane gas analysis apparatus. Expired air was collected over a period of 10 minutes in each case, except for the two periods, 10 and 20 minutes after epinephrin injection, when 6 minute periods were taken, because of the rapidity of change after giving the drug. The ventilation, basal metabolism and respiratory quotients were thus obtained from the readings on the gasometer and analyses on the Haldane apparatus. Blood pressures were measured by a mercury sphygmomanometer.

The tablet form of epinephrin (Adrenalin, Parke, Davis and Company) was used in each instance, because of the tendency of epinephrin solution to deteriorate on standing. Each tablet contained  $\frac{1}{40}$  grain epinephrin and for injection two tablets were mixed with a minimum of water, each patient receiving therefore 0.625 mgm. of epinephrin.

## RESULTS

The reactions to epinephrin obtained both in our obese patients and in normal individuals are shown graphically in figures 1 to 6. To facilitate comparison, all values are expressed in per cent above or below the basal level. Individual and composite curves are given for each group. The basal values for all factors are shown in tables 1 and 2. In the charts it will be noted that each reading is not placed 10, 20 or 30 minutes after the epinephrin injection, but a little later in each case. The individual tests were all started at these intervals,

but in order to account for the time needed for making the test, a point midway between the beginning and end of the period was

TABLE 1  
*Obese subjects*

Name	Age	Height	Weight	Over weight	Basal ventilation	Basal R.Q.	Basal metabolism	Basal pulse	Basal blood pressure
		cm.	kgs.	per cent*	liters per hour†		per cent		
N C	28	157	81	45	395	0.79	0	63	114/78
L V	28	170	107	66	385	0.72	-4	55	110/68
H. C.	18	159	103	92	374	0.78	-4	79	124/82
P	38	154	106	81	456	0.79	+26‡	64	150/96
R	37	156	109	89	351	0.72	+5	63	120/82
D A	26	165	114	92	448	0.81	+5	66	122/80
R P	38	162	88	43	337	0.75	-9	59	116/78

\* These figures were calculated from the Tables of Association of Life Insurance Directors and Actuarial Soc of America, New York, 1912, p 38

† The basal ventilation is expressed in liters per hour, uncorrected for barometer and temperature.

‡ This patient showed a slightly elevated metabolism on several occasions, a cause for which was not discovered. She showed no evidence of hyperthyroidism. She had been on an obesity diet for several months and was losing weight satisfactorily

TABLE 2  
*Normal subjects*

Name	Age	Height	Weight	Basal ventilation	Basal R.Q.	Basal metabolism	Basal pulse	Basal blood pressure
		cm.	kgs.	per cent		per cent		
J G	38	167	73	287	0.77	-11	64	98/64
J C.	45	169	63	461	0.80	-5	87	118/78
W B †	64	164	54	347	0.76	-19	67	118/56
H H	19	159	50	320	0.81	-9	63	108/64
G C ‡	20	164	46				69	108/78

\* Footnotes \* and † of table 1 apply also to table 2

† W B had recently recovered from lobar pneumonia accounting for his low basal metabolism.

‡ Metabolic studies were not completed on patient G C

taken as the average. Thus the readings of the 10 and 20 minute periods have been charted as at 13 and 23 minutes, while the remaining periods are all charted 5 minutes after the test was started. Figures

1 and 4 show the results in the obese subjects, figures 2 and 5 in the normal controls and figures 3 and 6 represent a composite picture of all results for comparison

The only striking and constant differences between the obese and normal group were in the behavior of the respiratory quotient and in that of the pulse rate

*Metabolism* The heat production in the two groups was not significantly different In the obese an average metabolism of 28.3 per cent above basal was reached in 30 minutes, while a height of 26.1 per cent was reached in the normal controls The heat production had not quite returned to its basal level at the end of two and one-half hours

*Ventilation* The rate of ventilation was calculated on the hourly basis and showed an abrupt rise after epinephrin, the highest level being reached in both normal and obese subjects 30 minutes after injection These figures showed no significant variation from one another, the average reaching 38.1 per cent above basal in the obese subjects and 42.2 per cent in the normals The control curves showed greater individual variations than the obese and the drop to a lower level was a little more rapid in the composite control curve It will be noticed that the relative increase in ventilation was greater in both groups than that in the metabolism

*Pulse rate* The pulse rate rose more rapidly and to a greater height in the obese It is of interest to note that the average basal pulse in the obese was 65 while it was 70 in the controls The highest level in each case was reached in 30 minutes after injection, reaching 40 per cent above basal in the obese and 26 per cent in the normal individuals In no case did the pulse rates reach the basal level, but stayed up higher in the obese throughout the duration of the experiments

*Pulse pressure* The pulse pressures showed wide individual variations in both groups of cases In one obese case the pulse pressure dropped considerably below the normal level However the average pulse pressures showed no significant variation from one another

The average systolic pressure rose more in the normal than in the obese, reaching 11.2 per cent above basal in the former and only 4.2 per cent in the latter in 10 minutes, while at the end of the 30 minute period it had reached 14.3 per cent and 7.7 per cent in each respectively

The diastolic levels showed no marked variations. The systolic and diastolic blood pressure variations are recorded in figure 6 and show no great variation from one another.

*Respiratory quotient.* The respiratory quotients reached their highest level in 10 minutes after injection, somewhat sooner than the other factors. The average rise in quotient in the normal subjects reached a level of 19.6 per cent above the basal, while in the obese subjects 11.2 per cent above basal was the highest figure reached. The composite curve for the obese is consistently lower than that of the normals during the entire experimental period and the individual curves more often go below the basal quotient than not.

The significance of this altered behavior of the respiratory quotient after epinephrin in obesity is not entirely clear. It indicates that the increase in metabolism is met by a relatively greater oxidation of fat and less of carbohydrate than in normal persons. The respiratory rate increased proportionately and equally in both the obese and normals. Whether this difference in quotients throws any light on the fundamental nature of obesity cannot be told at present.

The interpretation of the respiratory quotients in terms of percentages of food stuffs burned cannot be made with certainty at present. The literature gives conflicting data on the effect of epinephrin on the protein metabolism and work is now in progress in this laboratory to determine this effect. The most convincing paper is by Allan et al (9), who believe that epinephrin has a decided effect on protein metabolism.

#### CONCLUSIONS

- 1 The characteristic increase in total metabolism, pulmonary ventilation, and pulse pressure following epinephrin injection was found to be not of a significantly different magnitude in the obese than in persons of normal weight.
- 2 The respiratory quotient, on the other hand, seemed to rise definitely less in obese persons than in normal persons after epinephrin.
- 3 The pulse showed more acceleration in the obese than in normal persons.

## BIBLIOGRAPHY

- 1 Means, J H *Jour Med Res*, 1915, xxvii, 121 *Studies of the Basal Metabolism in Obesity and Pituitary Disease*  
Means, J H. *Arch Int Med*, 1916, xvii, 704 *The Basal Metabolism in Obesity*
- 2 Boothby, W , and Sandiford, I *Jour Biol Chem*, 1922, liv, 783 *Summary of the Basal Metabolism Data on 8614 Subjects with Especial Reference to the Normal Standards for the Estimation of the Basal Metabolic Rate*
- 3 Wang, C C , and Strouse, S *Arch Int Med*, 1924, xxxiv, 573 *Studies on the Metabolism of Obesity III The Specific Dynamic Action of Food*
- 4 Plaut, R *Deutsch Arch f klin Med*, 1922, cxxxix, 285 *Gaswechseluntersuchungen bei Fettsucht und Hypophysikerkrankungen*
- 5 Lauter, S *Deutsch Arch f klin Med*, 1926, cl, 315 *Zur Genese der Fettsucht*
- 6 Cannon, W B *Bodily Changes in Pain, Hunger, Fear and Rage* D Appleton & Co , New York, 1915
- 7 Tompkins, E H , Sturgis, C C , and Wearn, J T *Arch Int Med*, 1919, xxiv, 269 *Studies on Epinephrin II Effects of Epinephrin on the Basal Metabolism in Soldiers with "Irritable Heart," in Hyperthyroidism and in Normal Men*
- 8 Sandiford, I *Amer Jour Phys* 1920, l, 407 *The Effect of the Subcutaneous Injection of Adrenalin Chlorid on the Heat Production, Blood Pressure and Pulse Rate in Man*
- 9 Allan, F N , Dickson, B R , and Markovitz, J *Amer Jour Phys*, 1924, lxx, 333 *The Relationship of Phosphate and Carbohydrate Metabolism II The Effect of Adrenalin and Phloridzin on the Excretion of Phosphate*

## EDEMA

### II THE EFFECTIVENESS OF ULTRAFILTRATION FOR QUANTITATIVELY DETERMINING THE "FREE WATER" CONTENT OF BLOOD PLASMA, AND FOR ESTIMATING PHYSICAL-CHEMICAL CHANGES OF THE PLASMA PROTEINS IN EDEMA

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The physicochemical condition of the blood plasma in edema has been the subject of much interest due to the attention which Fisher's (1) writings have drawn to the rôle of the colloids. In connection with a discussion of the causes of defective water secretion by nephritic kidneys, Fisher suggested that "acid acts upon the tissues of the body including the blood and lymph. The increased hydration capacity resulting from this makes the tissues hold more water in combined form (maintenance of body edema) while at the same time it prevents water becoming 'free' in the arterial blood stream." A review of the evidence for and against this theory is entirely outside of the scope of this paper. Our interests regarding Fisher's theory are chiefly directed towards the researches recently published by Beckmann (2).

By a process of ultrafiltration Beckmann studied the "free" water content of the blood plasma of seven cases of edema with varying etiological factors. In some instances he obtained a diminished amount of ultrafiltrate. Beckmann assumes that the amount of ultrafiltrate obtained from certain of these patients was diminished because the plasma colloids were swollen and held part of the water of the blood in combination so that it was not obtained by the process of ultrafiltration. In other instances where he obtained an increased amount of ultrafiltrate, he assumes that a converse physicochemical change had occurred in the plasma proteins, i.e., they were shrunken, thus lessening their ability to combine water, which would result in an increased amount of free water in the blood plasma.

Beckmann used the method of ultrafiltration described by Ellinger and Neuschlosz (3) He also made quantitative determinations of the dry residue of the blood plasma and of the plasma proteins He assumes that quantitative differences in the dry residue may cause variation in the amount of ultrafiltrate delivered, independent of the shrunken or swollen condition of the plasma colloids and developed a formula to correct for this variation

Our purpose in undertaking this research is not to utilize the formula developed by Beckmann, but simply to determine whether measurable differences from the normal can be found in the amount of water obtained by ultrafiltration from the plasma of patients with varying types of nephritis and edema, and to determine whether these differences, if present, bear any relationship to quantitative variation in the plasma proteins Moreover, if in edema the plasma proteins become shrunken or swollen, such changes should be detected by using ultra-filters of graded permeabilities

#### METHOD

The details of the technique employed in the process of ultrafiltration and the contributions of pioneer workers have been discussed by Bechhold (4), more recently Zilva and Muira (5) have contributed to the method by a different process for standardizing membranes Cushny (6) in his researches directed toward determining whether or not the crystalloids of blood plasma exist in combination with colloids, reduced the high pressure advocated by Bechhold (1 to 20 atmospheres) to 21 cm of Hg

We employed the essential principles of the technique advocated by Beckmann, i.e., a pressure equal to 77 mm Hg, and the gel for making membranes which was a glacial acetic acid solution of parlodion Figure 1 illustrates the simple apparatus which proved entirely effective for producing and maintaining, at a constant level, low hydrostatic pressures The pressure is raised by establishing a siphon which is not interrupted during the experiment, so that if a lowering of pressure occurs in the system by a slight leak of air or the filtering through of a few cubic centimeters of fluid, it is immediately restored with water from the reservoir

The ultrafiltration sacs employed in the experiments of table 1, were made according to the technique used by Beckmann, i.e., paper Soxhlet extraction thimbles were impregnated with a glacial acetic acid solution of parlodion (DuPont) by suspending each with a loop of thread sewed through the upper part 1 cm from the top and filling with the gel The gel was of such consistency that complete impregnation (indicated by drops of gel falling from the outer surface of the sac) occurred in 3 to 5 minutes The excess of parlodion was allowed to drain off,

then the sacs were fixed in distilled water and washed in running water for 12 hours. When not in use they were kept under distilled water in the ice chest. The membranes were brought into the pressure system by mounting each on a perforated rubber stopper of slightly less diameter than the membranes. Short glass tubing, one end of which was connected to the pressure system, the other end passing

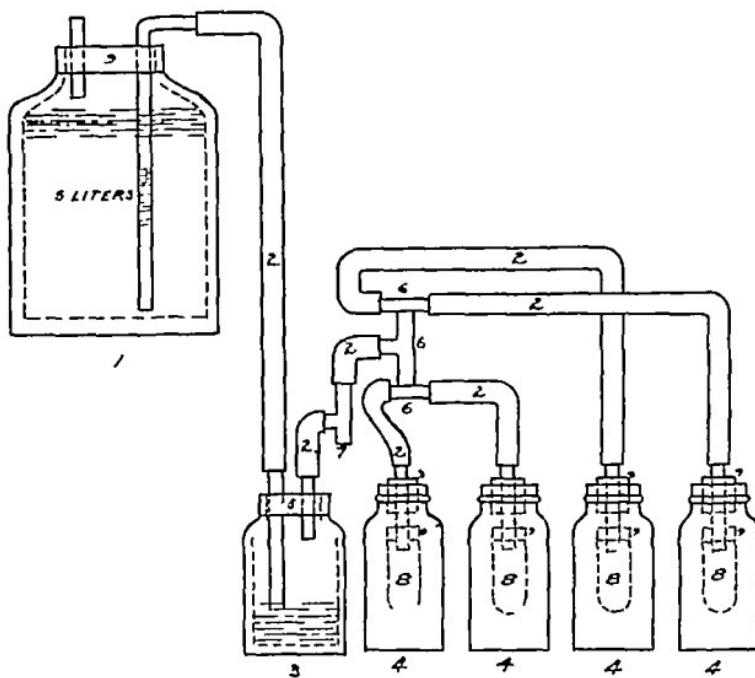


FIG 1 PRESSURE SYSTEM WITH MEMBRANES ATTACHED

1, cistern, 2, rubber tubing, 3, pressure bottle, 4, collecting bottles, 5, metal top, 6, "T" tubes, 7, "T" tube for manometer, 8, membranes, 9, rubber stoppers

through the perforated stoppers, admits the pressure from the system into the membranes. Above the filtration sac, the tubing passes through a large rubber stopper which loosely covers the receiving bottle. This prevents excessive evaporation of the ultrafiltrate, but admits atmospheric pressure to the outside of the membranes. The amount of blood plasma placed in each sac in all experiments was 5 cc. The duration of the experiment was 3 hours unless otherwise stated.

TABLE 1

*Showing variations in amount of ultrafiltrates obtained when unstandardized membranes are used*

Five cubic centimeters of blood plasma was placed in each ultrafiltration sac Time of each experiment = 3 hours Pressure 77 mm Hg (M) = male, (F) = female, (E) = palpable edema

Case number	Sac number	Ultra-filtrate cc	Protein in ultrafiltrate	Clinical diagnosis
1(Su)	2	0.8	0	Acute nephritis (E) (M)
	4	2.6	0	Acute nephritis (E)
	5	1.9	0	Acute nephritis (E)
	6	2.2	0	Acute nephritis (E)
10(G T)	17	2.3	++	Acute nephritis (F)
19 (C N)	30	2.45	0	Acute nephritis (E) (F)
	31	1.9	0	Acute nephritis (E) (F)
	32	2.0	0	Acute nephritis
	33	1.4	0	Acute nephritis
20 (C N)	34	1.35	0	Acute nephritis
	35	1.3	0	Acute nephritis
	36	2.0	0	Acute nephritis
	37	1.7	0	Acute nephritis (E) (M)
21 (J C)	38	1.8	0	Acute nephritis (E)
	39	2.2	0	Acute nephritis (E)
	40	2.0	0	Acute nephritis
	41	2.0	0	Acute nephritis
3 (S W)	7	2.0	0	Normal (M)
4 (A J)	8	1.1	0	Normal (M)
	9	1.3	0	Normal
5 (Y W)	10	2.7	0	Normal (F)
3 (F W)	5	2.4	0	Normal (M)
2 (B F)	4	2.6	0	Normal (M)
	6	2.6	0	Normal
14 (C J)	22	1.6	0	Normal (F)
16 (A J)	24	1.5	0	Normal (F)
5 (Y W)	11	3.4	+	Normal (F)
15 (F H)	23	1.4	0	Nutritional edema (E) (M)
11 (N N)	18	3.2	+	Diabetes (M)
	19	2.0	0	Diabetes
12 (N A)	2	2.0	0	Postpartum 8th day
13 (V C)	21	1.5	0	Postpartum 9th day
6 (H S)	10a	3.8	++	Postpartum 7th day
	25	1.8	+	Toxemia of pregnancy
17 (R B)	26	1.3	0	Toxemia of pregnancy
	27	1.3	0	Toxemia of pregnancy
18 (R B)	28	1.3	0	Toxemia of pregnancy
	29	1.8	0	Toxemia of pregnancy
6 (H S)	12	3.4	+	Postpartum 8th day
	13	2.8	0	Hypertension (F)
8 (E D)	14	2.8	0	Hypertension
	15	1.6	0	Hypertension
9 (D M C)	16	2.8	0	Chronic nephritis (M)

## RESULTS

The data presented in table 1 show the amount of ultrafiltrate obtained from the plasma of normal individuals, patients with toxemias of pregnancy, normal postpartum patients, and patients with chronic and acute nephritis with and without edema of cardiac or renal origin<sup>1</sup>. The only group of this series that shows any degree of constancy in the amount of ultrafiltrate obtained is the toxemia of pregnancy group. The most extreme degree of variation occurs among members of the other groups. Obviously, attempts to estab-

TABLE 2

*Variations in amount of ultrafiltrate obtained from the blood plasma of a dog, using different sacs*

The duration of the experiment in each case is 3 hours. Amount of plasma placed in each sac is 5 cc. Pressure 77 mm Hg. The ultrafilters are not standardized.

Sac number	Ultrafiltrate	Protein in ultrafiltrate	Percentile increase over least of this series <i>per cent</i>
	cc		
1a	3.5	++	41
2a	3.1	Very slight	25
3a	2.8	None	16
4a	3.3	None	37
5a	3.6	++	50
6a	2.4	None	0
7a	2.9	None	20
8a	3.0	Slight	25
9a	2.4	None	0
10a	3.0	None	25
11a	3.2	None	33
12a	3.6	++	50

lish the amount of free water available from the blood plasma of normal individuals, or to classify the findings from pathological sera in clinical groups on the basis of these results is entirely futile. Either the available water of the blood in conditions of health and in these

<sup>1</sup>The clinical cases studied in this report were all patients at the Cook County Hospital, Chicago, Illinois. We gratefully acknowledge our indebtedness to the internes and resident physicians of this hospital. Thanks are especially due to Dr H. A. Singer, whose cooperation made the clinical material used in this research available.

TABLE 3

*The influence of adsorption by the membranes in reducing the amount of ultrafiltrates obtained from membranes standardized but once, and used repeatedly*

The amount of plasma used, pressure and time, are the same as in tables 1 and 2. All studies excepting those marked \* were on blood plasma obtained from patients of the pathological obstetrical ward.

Date, 1925	Ultrafiltrate cc	Total proteins mgm per cc	Case number	Clinical diagnosis	Condition of ultrafilter
March 31	1 3	55	1 (R B )	Toxemia of pregnancy Slight palpable edema	Used several times
March 31	1 3		1 (R B )		Used several times
March 31	1 3		1 (R B )		Used several times
April 8	2 1	73	1 (R B )	No edema	Used once
April 8	1 25		1 (R B )	No edema	Used once
April 23	1 25	88	1 (R B )	Slight edema	Used several times
April 23	1 3		1 (R B )	Slight edema	Used several times
May 2	1 2	89	1 (R B )	No edema	Used several times
May 8	1 25		1 (R B )	No edema	Used several times
May 8	1 34		1 (R B )	No edema	Used several times
May 8	1 4		1 (R B )	No edema	Used several times
May 15	1 4	71	1 (R B )	No edema	Used several times
April 3	1 15	67	2 (O N )	Toxemia of pregnancy No edema	Used several times
April 3	1 2		2 (O N )	Toxemia of pregnancy No edema	Used several times
April 3	1 1		2 (O N )	Toxemia of pregnancy No edema	Used several times
April 21	1 5	82	2 (O N )	Toxemia of pregnancy No edema	Used several times
April 24	1 5		2 (O N )	Toxemia of pregnancy No edema	Used several times
April 24	1 45		2 (O N )	Toxemia of pregnancy No edema	Used several times
August 22	2 8	68	3 (E D )	Toxemia of pregnancy Marked edema	Used once
August 22	2 8		3 (E D )	Toxemia of pregnancy Marked edema	Used once
August 22	1 6		3 (E D )	Toxemia of pregnancy Marked edema	Used several times
August 22	2 8		3 (E D )	Toxemia of pregnancy Marked edema	Used once
March 25	1 3	56	4 (A B )	Toxemia of pregnancy No edema	Used several times

TABLE 3—Continued

Date 1925	Ultrafiltrate cc	Total protein mgs. per cc.	Case number	Clinical diagnosis	Condition of ultrafilter	
March 25	1 3		4 (A B )	Toxemia of pregnancy No edema	Used several times	
March 25	1 3		4 (A B )	Toxemia of pregnancy No edema	Used several times	
April 2	1 3	70	4 (A B )	Slight edema	Used several times	
April 6	1 0	74	4 (A B )	No edema	Used several times	
April 6	1 1		4(A. B )	No edema	Used several times	
April 6	1 2		4 (A B )	No edema	Used several times	
May 12	1 5	59	5 (B M )	Toxemia of pregnancy	Used several times	
May 26	1 0	61	6 (L W )	Toxemia of pregnancy	Used several times	
May 26	1 5	62	7 (E G )	Toxemia of pregnancy	Used several times	
June 6	1 0		7 (L G )	Toxemia of pregnancy	Used several times	
June 2	1 0		8 (L B )	Hypertension	Used several times	
July 10	2 5	72	9 (L C )	Normal	Used several times	
July 10	2 6		9 (L C )	Normal	Used several times	
July 10	2 8		9 (L C )	Normal	Used several times	
July 8	2 3	56	10 (A B )	Eclampsia	Used several times	
July 8	2 15		10 (A B )	Eclampsia	Used several times	
April 28	1 2	65	10 (V L )	Eclampsia	No edema	Used several times
April 28	1 1		10 (V L )	Eclampsia	No edema	Used several times
April 28	1 1		10 (V L )	Eclampsia	No edema	Used several times
May 6	1 1	67	11 (D E )	Eclampsia.	No edema	Used several times
May 1	1 1		12 (N N )*	Normal pregnancy		Used several times
May 1	1 3		12 (N N )*	Normal pregnancy		Used several times
May 11	1 7		13 (A A )*	Normal pregnancy		Used several times
May 1	1 1	98	14 (P B )*	Normal pregnancy		Used several times
April 13	1 6		15 (N P )*	Normal pregnancy		Used several times
April 16	1 6		15 (N P )*	Normal pregnancy		Used several times
April 18	1 3	68	16 (F F )*	Normal pregnancy		Used several times
August 25	1 8		17 (F U )*	Normal pregnancy		Used several times
May 20	1 4		18 (C J )*	Normal		Used several times
May 27	1 5		18(C C )*	Normal		Used several times
May 28	1 0		19 (A R )*	Normal		Used several times
May 29	1 3		20 (M. B )*	Normal		Used several times
June 30	1 2		21 (B E )*	Normal		Used several times
June 30	1 2		21 (B E )*	Normal		Used several times
June 30	1 2		21 (B E )*	Normal		Used several times
May 10	1 5		22 (E. G )*	Normal		Used several times
June 1	1 6		23 (L B )*	Normal		Used several times

diseases is exceedingly variable, or sufficient uniformity in the permeability of the membranes made according to the method described above, had not been attained.

This second possibility was investigated, and the results of an experiment planned to test the uniformity of the ultrafiltration sacs are given in table 2. In this test 12 sacs were used, 5 cc of blood plasma from the same dog was placed in each sac and the sac fitted to the pressure apparatus. At the end of 3 hours a 37 per cent variation was found in the total amount of protein-free ultrafiltrate delivered from the several sacs. Obviously this difference was due, not to a change in the "free" water content of the blood plasma of the dog, but to differences in the ultrafiltration membranes.

Recognizing the need of a more constant degree of permeability in our membranes, we attempted to eliminate variations in the thickness by using a gel (7 per cent glacial acetic acid solution of parlodion) of such consistency that the paper Soxhlet thimbles were completely impregnated in 2.5 to 3 minutes. They were then drained and dried for 8 minutes, immersed in the gel, drained again for 5 minutes, fixed in distilled water and washed as already described. The membranes were then standarized by placing 5 cc of distilled water in each and fitting into the pressure apparatus. Only those were chosen for use that delivered 5 cc of distilled water in 75 to 90 minutes, at a pressure of 77 mg Hg. Such thimbles delivered 2.8 to 3.0 cc of protein-free filtrate from 5 cc of ovaleted plasma from normal individuals or dogs in 165 to 180 minutes.

Table 3 shows the amount of ultrafiltrate obtained from a series of women, some of whom were in normal state of health, others were suffering from toxemia of pregnancy, or were normal postpartum patients. The membranes used in the experiments of this series were all standarized with distilled water and then tested with normal serum as mentioned above. After this, in most instances, they were used to test the amount of ultrafiltrate that could be obtained from the blood plasma of nephritics or patients with deferred diagnosis. They were then used in the series of cases reported in table 3. These data are important only in showing how consistently false results may be obtained because of adsorption by the gel of colloidal substances in the plasma.

TABLE 4

*Data selected from experiments in which standardised membranes are used*

Recalibration shows little or no change in the membranes during the experiment \* (M) = male, (F) = female. Time, pressure and amount of plasma are the same as in table I

Case number	Date 1925	Ultra filtrate cc.	Total protein in plasma mgm per cc.	Clinical diagnosis	Dis- tilled water deliv- ered after test cc.
1 (R. B.)	April 8	2.1	73	Toxemia of pregnancy Slight palpable edema	4.7
	April 8	2.1			4.7
2 (E. D.)	August 22	2.8	68	Toxemia of pregnancy Slight palpable edema	5
	August 22	2.8			5
3 (L. C.)	July 10	2.6	72	Toxemia of pregnancy	5
	July 10	2.8			5
4 (A. B.)	July 8	2.3	56	Toxemia of pregnancy	4.8
5 (Su)	July 14	2.15	63	Acute nephritis Marked edema (M)	4.7
	July 14	2.6			4.9
	June 18	2.6			4.9
6 (G. T.)	June 8	2.8	88	Acute nephritis	5
	June 8	2.7			5
7 (MII)	August 19	3.0	100	Chronic nephritis and per nicious anemia (M)	5
	August 11	3.1			5
8 (C. N.)	March 14	2.5	83	Acute nephritis. Edema (M)	4.8
9 (J. C.)	May 18	2.8	52	Acute nephritis. Edema (M)	5
	August 22	3.0	51		5
	September 29	2.7	60		4.4
	September 29	2.5			4.4
10 (L. D.)	August 20	3.0	62	Chronic nephritis (F)	5
11 (N. V.)	August 19	3.2		(M)	5

TABLE 4—*Concluded*

Case number	Date, 1925	Ultra filtrate cc	Total protein in plasma <i>mgm per cc</i>	Clinical diagnosis	Dis- tilled water deliv- ered after test cc
12 (C T )	August 24	3 1	68	Acute nephritis    Edema	5
	August 24	3 2			5
	August 24	3 1			5
13 (C B )	September 15	2 5	81	Hypertension (F)	5
	September 15	2 5			5
14 (Y W )	August 26	2 7		Normal (M)	5
	August 26	3 0			5
15 (H S )	August 31	3 4		Normal    Postpartum	5
16 (A J )	August 25	2 8		Normal	5
	August 25	2 8			5
17 (D H )	April 6	2 3		Normal (M)	4 7
	April 6	2 35			4 7

\* Protein was present in the ultrafiltrates of cases 10 (L D ) and 15 (H S )

Critical examination of the technique and further analysis and experimentation showed that the low values were due to lessened permeability of the membranes, and that these ultrafilters had changed during the process of using, and would no longer deliver 5 cc of distilled water in 90 minutes. This tendency to change in permeability makes it necessary to know the degree of permeability of the membranes at the end of each experiment as well as at the beginning. We find that the membranes usually become changed as a result of the first ultrafiltration of blood plasma, so that instead of delivering 5 cc of distilled water in 90 minutes, they deliver 5 cc of distilled water in two hours, therefore two hours becomes the standard for subsequent recalibration tests. The membranes may become "set" at this point, if so they may be repeatedly used without a measurable reduction in the permeability. But they must be tested after each using with distilled water, since changes may occur after the second or subsequent

using. When sufficient adsorption has occurred to reduce the permeability of the membranes about one-half they may remain remarkably constant for a number of experiments, or the permeability may slowly continue to decrease or suddenly increase. A calibration test made with distilled water reveals their condition.

Table 4 gives the results obtained from a series of 33 ultrafiltration experiments made with the blood plasma of 17 subjects, including normal men and women, patients with nephritis with and without edema and hypertension, and patients suffering from toxemias of pregnancy. These results are chosen from experiments where recalibration of the membranes with distilled water showed that the permeability of the membranes had remained relatively constant during the experiment. The data show that there is no significant difference in the quantity of ultrafiltrate obtained from these patients or normal individuals, regardless of the amount of edema or nephritis present.

#### DISCUSSION

The amount of ultrafiltrate obtained by filtering serum through membranes made according to the technique advocated by Beckmann, is of no significance unless the limits of effectiveness of the ultrafilters are definitely known. The permeability of the membranes depends largely upon the amount of gel entering into the composition and at present no method for *exactly* controlling this suggests itself.

Walpole (7) showed, by chemical analysis, that the amount of gel in adjacent 5 cm strips of membrane varies appreciably even where the membranes are made after the most accurate and painstaking method, and no paper support is introduced. Furthermore, micrometer measurements show considerable differences in the thicknesses of such strips. In the method used by Beckmann, no factor in the technique is controlled excepting the length of time taken to impregnate the paper Soxhlet thimbles, and that is given a leeway of 3 to 5 minutes. The paper thimbles vary at different points. This gives a base irregularly impregnated with gel and hence with differences in porosity at different points. Other conditions which we have found to cause changes which reduce the amount of ultrafiltrate are first, the degree of drying of the membranes before fixing in water, second,

swelling of the gel which occurs in some cases, after 12 hours of washing in water, so that membranes used only for experiments with distilled water deliver lessened amounts of ultrafiltrate after several days, third, exposure to drying during the preparation for an experiment, fourth, the adsorption of colloidal substances by the gel

The data submitted in table 2 show that uniform results cannot be obtained from blood plasma of the same subject where the above factors are operating in an unknown way. The 12 membranes used in the series of table 2 had not been previously used, so the 50 per cent difference found in the amounts of ultrafiltrate obtained from the same blood is due either to changes in the permeability that occurred during the process of making, or to differences in the amounts of adsorption which took place during the experiment.

Proper emphasis must be placed upon the *important factor of adsorption*. If our work had been interrupted at the point where the investigations of table 3 were completed, we could have advocated the false conclusion that the water available, by methods of ultrafiltration, from the blood plasma of normal women, postpartum patients and patients with toxemias of pregnancy, is 40 to 50 per cent less than that available from normal men. The results were obtained from membranes used several times without recalibration.

Comparable amounts of ultrafiltrate may be obtained from membranes of the same calibration, but recalibration is necessary after each experiment in order to make sure that the permeability has not changed during the experiment.

Standardized membranes, recalibrated, were used for the experiments given in table 4. These results indicate that the quantity of ultrafiltrate obtained in three hours with pressure of 77 mm Hg from blood plasma of normal individuals, postpartum patients (both uncomplicated and of the toxemia group), chronic and acute nephritis with and without palpable edema, does not vary to any significant degree. Further evidence supporting these results was obtained from the following experiments which differs from those previously reported, only in that the time for the ultrafiltration was extended until no more fluid was delivered from the membranes (9 to 15 hours). The blood plasma from 10 subjects was tested for "free" water content according to the process of ultrafiltration. The subjects included 3 patients with

chronic nephritis (2 had moderate edema), 1 with acute nephritis accompanied by marked edema, 1 with essential hypertension and 5 normal individuals Twenty ultrafiltration experiments were made in this series, using standardized and recalibrated membranes The total amounts of ultrafiltrates delivered from the 5 cc portions of plasma varied from 4.3 to 4.7 cc Only one sac delivered as small an amount as 4.3 cc and on recalibration with distilled water, the permeability of this sac was found to be markedly reduced We believe the slight differences in the amounts of ultrafiltrate delivered by the other sacs to be due to undetectable changes in the permeability of the membranes We do not offer these results as evidence that the available water of the blood plasma may not vary under some conditions We believe, however, that such changes cannot be detected by this method

Ultrafiltration, according to Bechhold, is not a method for detecting the *amount of water available*, but for separating colloidal particles of different sizes The two processes are no doubt closely related, but not identical For example, membranes of a given porosity will hold back all colloidal particles above a certain size regardless of the amount of fluid filtered through, the lower limit of permeability for a given colloidal particle is well defined In measuring water the degree of refinement in the technique is infinitely more difficult, because molecules of water filter through when the porosity is entirely too small to pass other substances found in the blood plasma If 5 cc of distilled water is placed in a membrane of diminished porosity, only a small amount of ultrafiltrate is obtained at 77 mm Hg and 3 hours time If the same amount of water is placed in a membrane of slightly greater porosity, it will completely filter through (length of time and pressure as above) Using the same membranes and reducing the pressure to 33 mm Hg (approximately the hydrostatic pressure of the capillaries) only 80 per cent of the distilled water passed through at the end of 9 to 15 hours Obviously this is not a test of the amount of free water in the contents of the filters, but a failure in some cases of the gel membranes to give rapid passage to free water under a given hydrostatic pressure because of the fineness of the pores The same condition holds to a great degree in experiments with blood plasma

We find no indication of marked physico-chemical changes in the

plasma proteins in cases of nephritis with or without edema, at least as indicated by the ultrafiltration of the plasma. Results in table 4 were obtained by standardized membranes. No protein molecules pass through where the quantity of ultrafiltrate measures 2.8 to 3.0 cc or less. If the permeability of the membranes is but slightly increased so that the amount of fluid delivered is increased by 0.4 cc the ultrafiltrate gives a slight positive protein test<sup>2</sup> in all conditions tested. It does not seem probable that changes in size of the colloids through this small range could account for the binding of water by the blood protein described by Beckmann. However, one must consider the possibility of various proteins of the blood swelling differently. Hydrostatic pressure equal to 77 mm Hg used in Beckmann's and my own experiments may tend to overcome the power of the slightly swollen colloids to hold water, hence at such pressure slight differences in amount of ultrafiltrate due to the hydration processes of the colloids may not be detected.

The nature of the proteins in solution influences the amount of ultrafiltrate delivered much more than do slight quantitative variations found in blood plasma. For example, 5 cc of a 7 per cent solution of gelatin yields only about 0.7 cc of ultrafiltrate from the standard membrane (pressure and time as in table 1), whereas a 7 per cent solution of colloids as found in blood plasma yields 2.8 to 3.0 cc.

#### SUMMARY

1 The amount of ultrafiltrate passing through a gel membrane in a given time and at a given pressure is of little significance unless the permeability of the membrane is definitely known.

2 Gel membranes should be standardized both before and after each experiment to detect changes which may occur during the experiment.

3 Ultrafiltration does not measure the free water of the substance filtered, but the speed with which the membrane can give passage to free water under a given hydrostatic pressure.

4 When standardized membranes were used in a system with a hydrostatic pressure equal to 77 mm of Hg the amount of ultrafiltrate

<sup>2</sup> Nitric acid ring test (Heller)

obtained in three hours did not vary significantly whether obtained from the blood plasma of normal men and women or of patients suffering from nephritis, with or without edema, or toxemias of pregnancy, or of postpartum patients without complications.

5 We failed to find, by this means, evidence either supporting or refuting the theory that swelling or shrinking of the plasma colloids may occur to a degree sufficient to influence the free water content of the blood plasma in patients with and without edema.

#### BIBLIOGRAPHY

- 1 Fischer, M H. Edema and Nephritis John Wiley and Sons, N Y , 1921,  
3rd ed
- 2 Bechmann, K. Deut. Arch Klin Med., 1924, cxlv, 22 Über das Blutodem  
Ultrafiltrationsversuche am Blutserum Ödematoser
- 3 Ellinger, A , and Neuschlosz, S M Biochem Zeitsch , 1922, cxxvii, 241  
Vergleichende Untersuchungen über Viscosität und Ultrafiltrationsge-  
schwindigkeit von Serum.
- 4 Bechhold, H., and Bullowa, J G M Colloids in Biology and Medicine. D  
Van Nostrand and Co , N Y , 1919
- 5 Zilva, S S and Miura, M Biochem. Jour , 1921, xv, 422 XLVIII The  
Differential Dialysis of the Antineuritic and the Antiscorbutic Factors
- 6 Cushny, A. R Jour Physiol , 1919, lvi, 391 The Colloid Free Filtrate of  
Serum
- 7 Walpole, G S Biochem Jour , 1915, ix, 284 XXVI Notes on Collodion  
Membranes for Ultrafiltration and Pressure Dialysis



# DEPENDENCE OF THE FORM OF THE ELECTRO CARDIOGRAM UPON THE SITE OF MECHANICAL STIMULATION OF THE HUMAN VENTRICLES

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The relation of the form of the electrocardiogram to the site of stimulation in the ventricles still remains an open question, although patients have been studied from this point of view and many experiments on animals have been performed Einthoven (1) in 1908 was the first to attempt to localize the site of origin of ventricular premature contractions by an analysis of patients' electrocardiograms Even at the present time such localization cannot be made with any degree of accuracy in the human heart

## LITERATURE

Early investigations on the site of origin of ventricular premature contractions as ascertained by the electrocardiogram were abstracted in 1913 by Rothberger and Winterberg (2), we have used their paper in part as a guide for the following review of the literature Einthoven (3) was the first to direct attention to the atypical complexes of ventricular premature contractions in man Later, in 1908, he (1) was again the first to attempt the localization of the site of origin of ventricular premature contractions by studying the direction of the deflections in relation to the three axial leads. He employed Lead I to distinguish between the right and left sides, and the other two leads to differentiate proximity of the point of origin to base or apex. One year previously Kraus and Nicolai (4) had reported the results of their experiments on direct stimulation of the right and left ventricle of the dog's heart they obtained oppositely directed diphasic complexes by the stimulation of these two chambers. For a time they considered such diphasic electrocardiograms as possibly due to hemisystoles but subsequently abandoned this theory as untenable. Nicolai and Rehfisch (5) supplemented the previous work by showing that the greatest contrast in electrocardiograms resulted from the stimulation of the extreme right of the base as compared with that of the point

farthest left of the apical region. They also found that every point stimulated gave a characteristic electrocardiogram more or less approaching in form the foregoing extreme types. In 1910, Kraus and Nicolai (6) divided the electrocardiographic curves of ventricular premature contractions induced experimentally in dogs' hearts into three fundamental types: type A, which resulted from stimulation of the apex; type B, of the base; and type C, polyphasic curves obtained by stimulating a central, more or less horizontal zone, situated about midway between apex and base. Nicolai (7) stressed the contrast between electrocardiographic curves from stimulation of base and apex, whereas Kahn (8) found the difference to depend upon which ventricle, right or left, had been stimulated. Subsequently Kahn (9) extended his experiments to the dorsal surface of the dog's heart, and found that in this region also, contrasting electrocardiograms depended not upon stimulation of base as compared with apex, but upon right as compared with left ventricle.

Rothberger and Winterberg (10) (1910-1911) found that there are exceptions to the general principle that ventricular premature contractions always yield anomalous complexes. They showed that stimulation of a point along the interventricular groove in a line connecting the anterior border of the right auricle with the apex, gave electrocardiograms which were normal in form. This observation, which has been confirmed, they explained by suggesting that from the point artificially stimulated the impulse traveled along abnormal pathways and reached both ventricles, causing them to contract simultaneously, just as they do as the result of a normal impulse conducted along normal channels. In 1915, as a result of the inability to localize with certainty the site of origin of ventricular premature contractions in man, the same authors (2) undertook to investigate the whole question again experimentally. For this purpose they selected dogs, and employed two leads, namely the standard Lead I and an anus-oesophagus lead which corresponds in a general way to standard Lead III. Their results are most easily seen by studying the diagram which illustrates their paper. Translating their language, for purposes of description only, into terms introduced by Lewis, one may say the following. On stimulation of a greater portion of the right ventricle, a concordant dextrocardiogram was obtained, that is to say, the first main deflection in both leads was directed upward. Similarly the greater portion of the left ventricle, that is most of the apex and dorsal surface, when stimulated yielded a concordant levocardiogram, that is to say, the first main deflection in both leads was directed downward. This result is what might be anticipated and is in harmony with the results of other investigators. But they found two unexpected exceptions to the general rule. In the first place, a basal portion of the left ventricle situated immediately beneath the left auricular appendix yielded an electrocardiogram which for descriptive purpose we may call a discordant dextrocardiogram, that is to say, the first chief deflection in Lead I was inverted, but in the anus-oesophagus lead was upright. In the second place, a somewhat similar exception was found in the right ventricle involving its apical and dorsal

portions, which yielded curves of the discordant levogram type, that is to say, the chief deflection in Lead I was upright but in the anus-oesophagus lead was inverted. The boundary on the ventral surface of the heart between the two areas yielding dextrocardiograms and levocardiograms respectively in the anus-oesophagus lead, was not a sharp line but a broad zone within which intermediate electrocardiograms with relatively small deflections were obtained. The location of this belt corresponds in general with that found by Kraus and Nicolai (6), it does not coincide with the interventricular groove, but is more horizontal in direction than the latter and is situated somewhat nearer the apex than the base. It is clear from these exceptions that the type of electrocardiogram obtained is not wholly dependent upon the particular ventricle, right or left, to which the artificial stimulus has been applied. Rothberger and Winterberg offer no final explanation for these unexpected results, and emphasize the fact that their findings cannot be applied to the human heart. Their findings are interesting and will be discussed after our results on the human heart have been given in detail.

Further experiments were carried out in this field by Lewis (11, 12). He concluded that when the surface of the ventricles is artificially stimulated, the excitation wave spreads radially in all directions, pierces the thickness of the ventricular wall, reaches the specialized conducting system, and thus is propagated at a greater velocity along the so-called conducting network to all parts, first of the corresponding ventricle, and ultimately of the opposite ventricle. Lewis reached this conclusion by stimulating a number of points in series on the surface of the two ventricles, recording the electrocardiograms and comparing the distance from the point stimulated to the underlying conducting network with the interval from the time of the stimulus to the first chief phase of the response. According to Lewis the "form of the electrocardiogram yielded by stimulating the surface of the ventricle seems to depend upon two chief factors, the relation of the point stimulated to the two networks of Purkinje, and its relation to the mass of ventricular muscle as a whole. Of these two factors the first exerts the dominant influence. The form naturally changes profoundly with the lead."

The experimental production of premature contractions in man by direct mechanical stimulation has heretofore been studied electrocardiographically only by August Hoffmann (13, 14). Like us, he studied patients who had been subjected to rib resection for empyema and in whom the heart on the left side was covered only by the soft tissues. He stimulated the heart both with galvanic current and with a percussion hammer. Unfortunately the electrocardiographic curves he has published are so poorly reproduced that a satisfactory interpretation of them is impossible. He states, however, that the electrocardiogram yielded by the stimulation of various points was always the same the chief deflection being inverted in Lead I, and upright in Lead III, and similar to that resulting from a right bundle branch lesion. There is an apparent contradiction in these two statements. He concluded that the mechanical stimulus on the surface of the ventricle was always propagated to the same specially susceptible centre, probably

in the left branch of the Tawara system, and from there spread to the left ventricle along the specialized conducting system

#### SUBJECT OF OBSERVATIONS

An opportunity has presented itself to investigate the subject in a patient whose ribs on the left side had been removed in the course of a modified Estlander operation for empyema some twenty-five years before. Over the left lateral chest wall the contractions of the auricles and ventricles are distinctly seen and felt though the soft tissues. By appropriate mechanical stimulation premature contractions can be elicited from either ventricle or auricle. We are reporting in this paper the results obtained by such artificial stimulation, more particularly of the ventricles.

*Clinical history* H L is a man aged 60 years. He was admitted to the Mt Sinai Hospital on June 1st, 1925<sup>1</sup>. During the favorable months of the year he exhibits himself as "The Human Heart Wonder". The history and physical examination in so far as they concern our study is as follows. The family history is unimportant.

*Past history* Twenty-five years ago he suffered from an attack of pneumonia which was followed by left hydropneumothorax and subsequently by empyema of the left pleura. One year after this illness a simple thoracotomy was performed for the relief of empyema, this was followed by a second operation in which resection of the fourth, fifth, sixth, and seventh ribs of the left side was performed, later at a third operation the remaining ribs of the left side are said to have been resected. The sinus of the empyema wound drained for one year. Six years ago he had an attack of influenza. Since then he has been the subject of chronic heart block. There has been only one attack of syncope in this connection. He was readmitted to the Hospital for further study on January 1st, 1926 and was discharged on February 23rd, 1926.

*Physical examination* The patient was very emaciated. His weight was 96 lbs. The left chest wall was sunken as a result of the resection of the lateral portions of the second to eighth ribs. Regeneration of the ribs had not occurred. There was marked scoliosis of the spine, with convexity to the left. The left hypochondrium bulged prominently. Anteriorly over the left chest wall auricular contractions were distinctly seen over an area about 2 cm in diameter, opposite to and to the left of the second costal cartilage. The auricular sounds were audible over this area. Very forcible ventricular contractions could also be seen and felt through the overlying soft tissues from the level of the upper border of the third

<sup>1</sup> We wish to thank Dr S Calvin Smith for his courtesy in referring this patient to us

rib to the level of the stump of the sixth rib. Cinematographs were taken to show these motions. A systolic murmur was heard over the area occupied by the ventricles. The auricles and ventricles were contracting independently. There was an occasional spontaneous ventricular premature contraction. Both auricular and ventricular premature contractions could be artificially induced with a percussion hammer by mechanical stimulation of the overlying skin. Mechanical stimulation of the auricles gave the patient a "stinging sensation inside the chest," but there was no sensation whatever when he was tapped over the region of the ventricles. There was marked arteriosclerosis of the radial and brachial arteries. The systolic blood pressure was 170 mm of mercury and the diastolic 75 mm. The percussion note over the right chest was hyperresonant. There was bronchovesicular breathing at the right apex. In the axillary region over the collapsed lung the breath sounds and many moist rales were heard. In fluoroscopic examinations of the chest<sup>2</sup> the left ventricle was not seen distinctly but was obscured by the spine and the ends of the resected ribs. It was seen only slightly on deep inspiration. Two or three movements of the auricles occurred to one of the ventricles. The right border showed faint auricular contractions in the intervals between systoles of the right ventricle. The right ventricle showed a slow contraction of wide amplitude, followed by a gradual relaxation, the shadow receding to the right and showing the auricular effect. An x ray photograph of the chest showed extreme collapse of the left lung and pleura. The heart was displaced toward the right side. The structural details of the left lung were obscured by the thoracic deformity.

*Diagnosis.* There was chest deformity due to a thoracoplasty operation, heart block of six years duration of unknown cause, arteriosclerosis and arterial hypertension (wide pulse pressure).

#### METHODS

It seemed possible that information might be gained by studying in this patient the relation of the form of ventricular premature contractions to the location of the point stimulated. The heart to the left of the sternum was covered by only the pericardium and soft tissues. It was possible to elicit ventricular premature contractions by tapping mechanically on the external chest wall overlying the heart, the mechanical stimulus being transmitted to the ventricular surface. We obtained electrocardiograms of ventricular premature contractions by stimulating eight points on the chest wall overlying the heart and have studied the resulting curves. Four points were selected which

<sup>2</sup>We wish to thank Dr Harry Wessler for making the fluoroscopic and x ray examinations in this patient.

were as far to the left as possible, four other points were chosen which lay along the left sternal margin as far as possible toward the midline. These eight points were marked with silver nitrate. Small squares of court plaster were placed over the points when the chest was photographed (fig 1). They were numbered 1 to 8 in a clockwise direction, beginning at the upper left hand. All of them lay over the ventricular surface of the heart. In as much as complete heart block was present (fig 1, point no 9) independent auricular and ventricular contractions could be seen distinctly so that the dividing line between auricles and ventricles could be located without difficulty. Point no 9 was just medial to the left nipple, over the auricular portion of the heart.

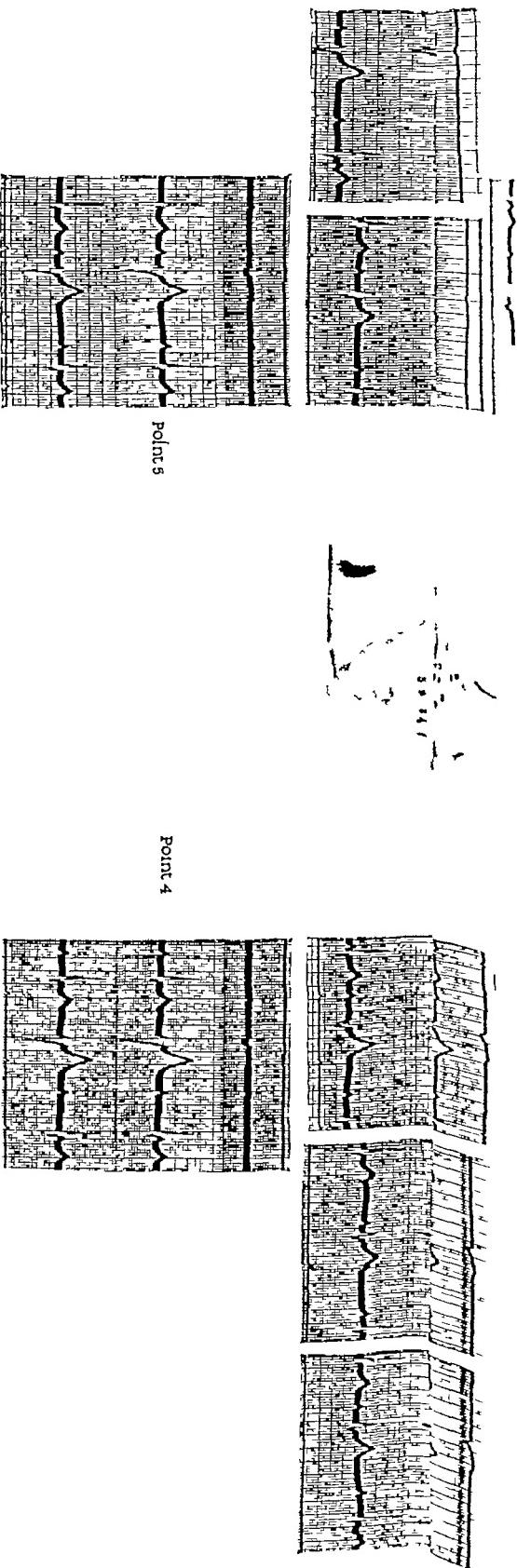
A triangular shaped rubber reflex hammer was used to tap the points. The patient lay flat in bed while observations were being made. The three standard leads of the electrocardiogram were photographed simultaneously by three galvanometers during mechanical stimulation of the heart. The arrangement of the galvanometers and the precautions taken in making the simultaneous electrocardiograms were those described by Cohn (15). The shadow of an electromagnetic signal indicated on the electrocardiographic record when the chest was tapped. One complete series of observations was made while the patient was lying flat, and a second, when he lay on his right side so that points nos 1 and 4 could be tapped again. By this manoeuvre we thought, provided the heart were freely movable, other points on the left ventricle would be exposed for stimulation. In a third series the patient was turned so that he lay on his left side and points nos 5 to 8 tapped. The plan was to expose if possible other points on the right ventricle. Electrocardiograms of many ventricular premature contractions were obtained from each point during each series of observations. The observations were repeated several times on the same day, on successive days, and also after an interval of several months. It is important to state that the same results were always obtained on tapping the same point.

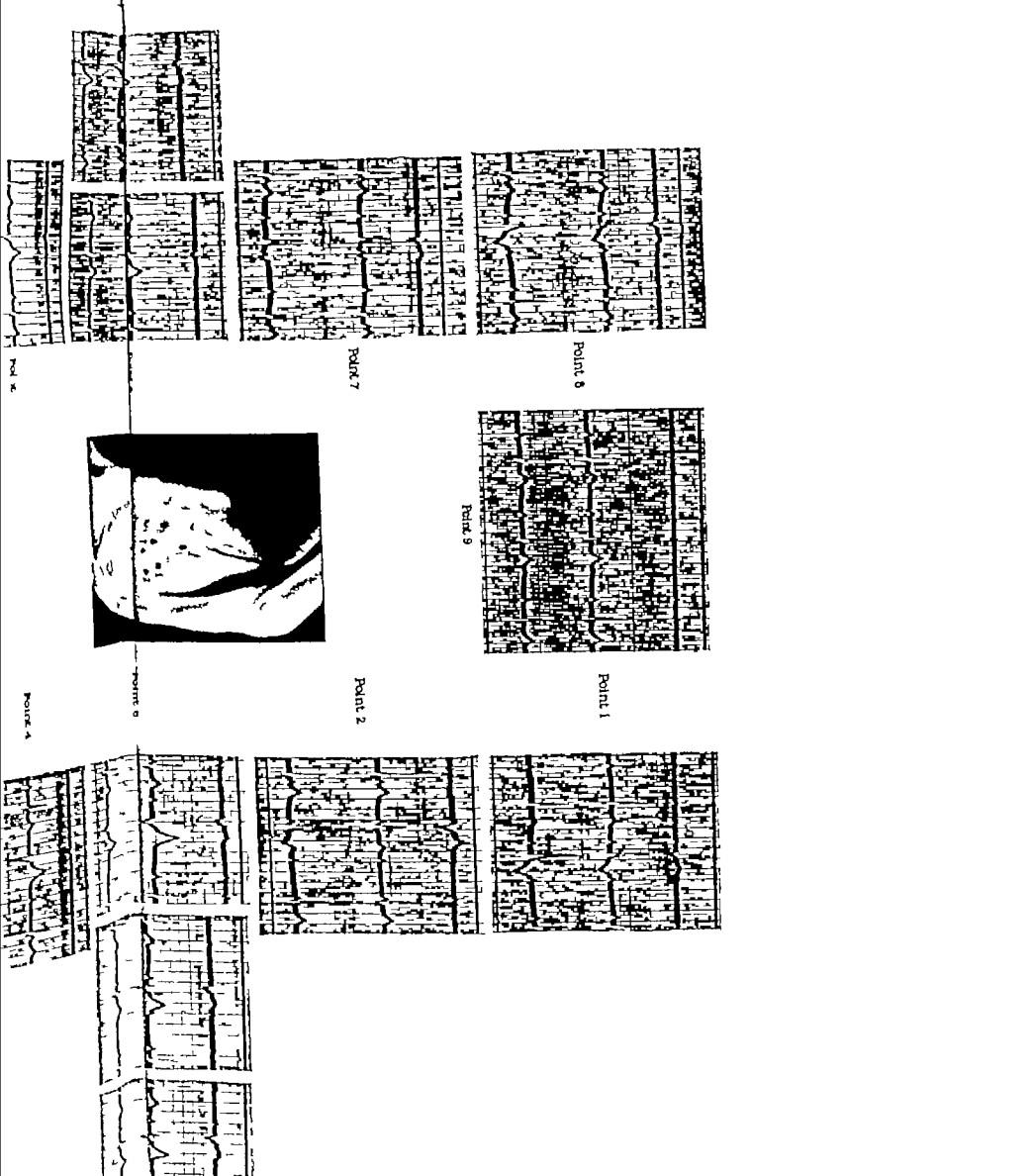
#### THE DATA

Premature contractions having the ventricular form were elicited from points nos 1 to 8 (fig 1). The ventricular premature contrac-



FIG 1 In the center of this figure is reproduced a photograph of the chest of the patient to show the position of points nos 1 to 9. Points nos 1 to 8 lie over the ventricular and point no 9 over the auricular part of the heart. On the right are reproduced typical electrocardiograms derived by stimulating each of the points on the extreme left of the patient's chest. On the left are reproduced representative electrocardiograms derived by stimulating each of the points along the sternal margin (points nos 5 to 8). At the top is shown an electrocardiogram obtained by stimulating point no 9 over the auricles. Points nos 5 to 8 lie along the left sternal margin, point no 5 being in the fifth interspace, point no 6 on a level with the upper half of the stump of the fifth rib, point no 7 in the fourth interspace, and point no 8 on a level with the upper half of the stump of the fourth rib. Points nos 1 to 4 are in the position indicated in the photograph. The distances of points nos 1 to 8 from the midsternal line are as follows, beginning with point no 1 8.7, 11.0, 11.5, 13.5, 11.0, 9.3, 8.0 and 7.2 cm. Point no 9 has the position indicated. The electrocardiograms from above downward read Leads I, II and III. The three leads are taken simultaneously by three galvanometers. Divisions of the ordinates equal  $10^{-4}$  volts. Divisions of the abscissae equal 0.04 of a second. The record of the electromagnetic signal at the top of each curve indicates when the point was tapped. The original curves are sharply contrasted black and white, no half tones are lost by the method of reproduction here used. The electrocardiograms are reduced to one-third of their natural size.





Point 6

Point 1

Point 9

Point 2

Point 7

Point 5

Point 8

Point 4

Point 10

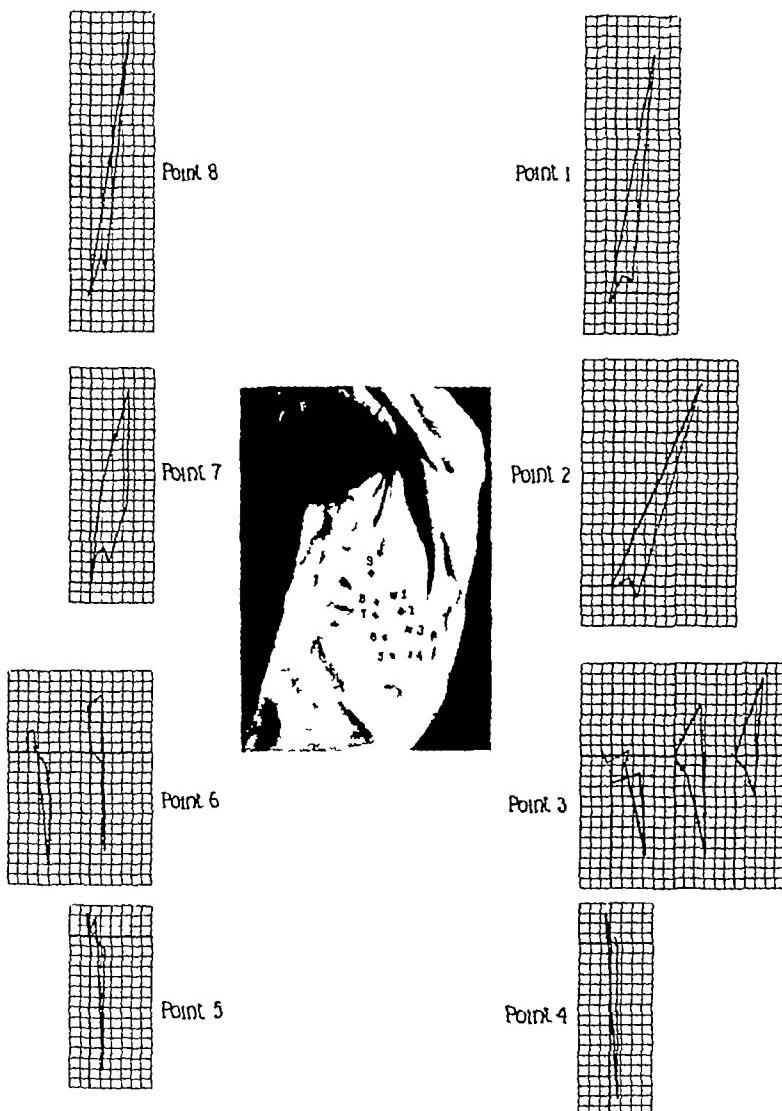


FIG 2 In this figure are presented monocardiograms derived from the ventricular premature contractions reproduced in the electrocardiograms in figure 1, points nos 1 to 8. The position of any monocardiogram in this figure corresponds with that of the complex from which it was derived in figure 1. The location of the points on the chest wall is shown in the photograph in the centre of the figure Natural size

Since in these records the three leads of the electrocardiogram were photographed simultaneously they were in phase and the measurements required no trial and error adjustment to bring about an arrangement such that Lead II equals Lead III plus Lead I. The monocardiograms derived from the ventricular premature contractions

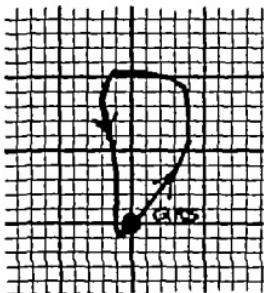


FIG 3

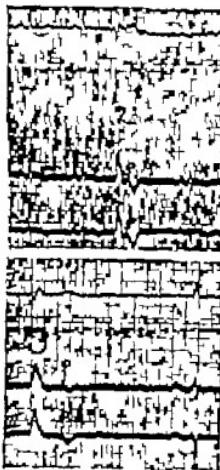


FIG 4

FIG 3 In this figure are presented the monocardiograms derived from the ventricular premature contractions reproduced in figure 4. The upper monocardiogram is derived from complex no 2, figure 4, and the lower curve from complex no 3 Natural size

FIG 4 In this figure two electrocardiograms from Prof Einthoven's patient are reproduced (Leads I, II and III from above downward). The ventricular premature contraction in both instances was obtained by tapping over the right ventricle. The three leads are taken synchronously. Divisions of the ordinates equal  $10^{-4}$  volts. In the upper curves (2) divisions of the abscissae equal 0.04 of a second, in the lower curves (3) 0.02 of a second. The original curves are sharply contrasted black and white, no half tones are lost by this method of reproduction. The electrocardiograms are reduced to one third of their natural size

in this series are of three types (fig 2) Those from points nos 1, 2, 7 and 8 are situated in the upper right hand quadrant from the starting point, the "centre of negativity" traveling in a clockwise direction, those from points nos 4 and 5 lie in the lower right hand quadrant for the most part and the "centre of negativity" travels in a counter-clockwise direction Those from points nos 3 and 6 are sometimes in one quadrant, sometimes in the other, and if constructed from ventricular premature contractions of the transitional variety they lie in both quadrants (fig 2) Monocardiograms do not however aid us in the interpretation of the data from the point of view of the origin of the premature contractions, although they help us to visualize the progress of events during the course of the various cardiac cycles The monocardiograms (fig 3) derived from the right ventricular premature contractions from Professor Einthoven's patient (fig 4) (see page 598) are similar in general to those obtained by us from point no 8 in our patient (fig 2)

Attention is called to the observation that in the electrocardiograms the R-R interval from the induced ventricular premature contraction to the following restored cycle is about 0.08 of a second longer than the normal R-R interval in this patient The delay is probably due to the time it takes the artificial stimulus to pass through the ventricular wall

#### DISCUSSION

Ventricular premature contractions may theoretically have arisen in any one of four ways (1) from the right ventricle, (2) from the left ventricle, or (3) from neither ventricle regarded as a lateral half of the heart, but rather as arising from the base or (4) from the apex, in this way assuming a division of the heart in cephalo-caudal halves According to the first view, major deflections directed upward in Lead III are derived from the right ventricle (VPCR), according to the second, they are likewise directed upward in Lead III, but are now believed to be derived from the left ventricle (VPCL) There are others who think that the form of ventricular premature contractions in which the major deflection is upward in Lead III is dependent not upon derivation from either the right or left ventricle, but rather upon an origin (3) from the base or (4) from the apex of the heart



FIG. 5 In this figure is reproduced a photograph of the left side of the chest of the patient. The patient was lying down. The absence of a bony chest wall to the left of the sternum is seen. The positions of points nos. 1 to 8 are indicated.

Lewis (12) thought that it was unwise to transfer the conclusions from his experiments in dogs to the form of ventricular premature contractions arising in the human heart, because of differences in distribution of the conduction system and in the relation of the heart to the chest wall in the two species. The ideal method in which to study the problem would be to stimulate the fully exposed heart. One could then force beats from base or apex, from right and left ventricle. The correlation between beat and curve could then be made directly as in the case of the dog, not inferentially as is necessary in studies of the type we are now reporting. Since the heart in this patient was not exposed to view we are unable to state the location of all the points stimulated. The problem then is this, is a given curve representative of an impulse originating in one of the ventricles or has its form an apex-base significance? Obviously a decision can be reached if the issue were based on a right-left ventricle difference, for here there is an anatomical division, in the apex-base case, there is none. It is of no value to approach the apex-base problem until proof is at hand to show that it is not a two-ventricle problem. It is necessary therefore to solve the two-ventricle problem first. This should be possible, provided it is practicable to ascertain the position of the anterior interventricular groove. If it turns out to be impossible to do this convincingly, then it is possible to do no more than to present the data which we have found as probabilities affecting the two-ventricle relation, the apex-base problem must remain in abeyance until more suitable methods become available.

Two of the points can however be located without much doubt. Point no 8 lay over the right ventricle near the base (figs 1 and 5), point no 4 must have lain over the left ventricle. Point no 8 was a short distance from the midsternal line (The distance from the midsternal line curving around the stump of the rib to this point was 72 cm). From a study of illustrations of the heart in anatomical atlases and also as the result of manipulation of gross anatomical specimens to learn what positions could be assumed by the heart on rotation, we concluded that it was hardly possible for the left ventricle to lie under this point. Point no 4 must have been over the left ventricle (figs 1 and 5). It was to the left of the point of maximal impulse and was 13.5 cm from the midsternal line in the fifth inter-

space. The chest began to curve posteriorly here so that this point must have been on the left lateral border of the heart. It is not possible to think of the heart in a position in which the right ventricle could have assumed this location. We can be fairly certain then that point no 8 lay over the right ventricle near the base and point no 4 over the left ventricle near the apex.

If we interpret this patient's curves according to the right-left ventricular origin theory, the premature contractions obtained from points nos 1, 2, 7 and 8 were derived according to the first theory from the *right* ventricle, those from points nos 4 and 5 were derived from the *left* ventricle, while those from points nos 3 and 6 were derived from an intermediate position. In accordance with the second theory, still on the basis of the two-ventricle theory, those premature contractions from points no 1, 2, 7 and 8 were derived from the *left* ventricle, and those from points nos 4 and 5 from the *right* ventricle and those from 3 and 6 would again be transitional. According to the apex-base theory of origin, the curves obtained from points nos 1, 2, 7 and 8 would be basal in origin, those from points nos 4 and 5 apical, and those from points nos 3 and 6 transitional. The fourth possible position, that curves from points nos 1, 2, 7 and 8 are apical in origin, is held by no one.

In this patient there was a sharp and constant zone on the surface of the chest and therefore on the heart lying beneath, above which ventricular premature contractions of one form were obtained and below which those of an opposite form, while along the zone those of a transitional type were elicited. A line drawn from point no 3 to point no 6 approximately marks the zone from which the transitional curves were obtained (fig 1). The problem now narrows itself to this question: could this zone cover the position of the interventricular septum? If it can, points nos 1, 2, 7 and 8 are obviously located on the right ventricle, and points nos 4 and 5 on the left, the transitional points nos 3 and 6, on intermediate positions too difficult to assign under these conditions.

We have attempted to arrive at information on this point in several ways. Physical examination yielded no information. The patient's heart was next subjected to careful fluoroscopic examination, but as was to be expected the interventricular septum could not be located.

Small lead discs were then placed on the points nos 1 to 8, and x-ray photographs were made of the chest. This procedure likewise yielded no useful information because all of the lead markers were projected almost in the same sagittal plane in the postero-anterior view and because the deformity of the chest confused the shadow of the heart in a lateral view. Since none of these examinations were of assistance in deciding upon the position of the interventricular septum, we turned

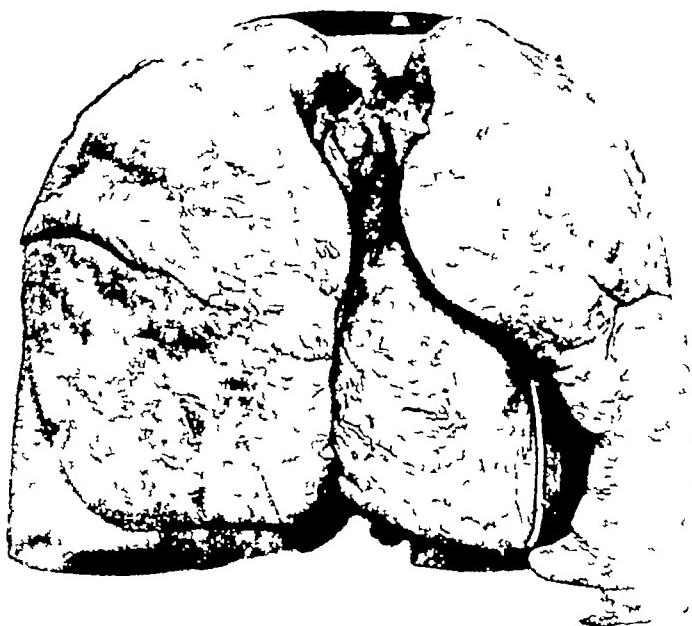


FIG 6 In this figure is presented a photograph of a normal heart and lungs removed from the chest *en masse* after thorough hardening. The view is anterior. The object is to show the position of the interventricular sulcus along which a white wire was placed. The left lung is to the reader's right.

to the examination of gross anatomical specimens. We were fortunate in finding a specimen which was suitable for our purpose.<sup>4</sup> The thoracic contents of a cadaver which was thoroughly fixed and hard-

<sup>4</sup>The specimen was loaned to us by Dr Adolf Elwyn of the Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York. We wish to thank Dr Elwyn for his courtesy in placing this specimen at our disposal.

ened *in situ* was removed from the chest *en masse*. There appeared to be no deformity in the position of the heart nor in its relation to the lungs (fig 6). A white wire was placed along the interventricular sulcus, in order to give the approximate position of the interventricular septum. Instead of photographing the specimen as one would normally and naturally do from in front, the optical axis being antero-



FIG 7 A left lateral view of the left normal heart and lungs shown in figure 6 is reproduced in this figure. The left lung is held aside in order to photograph the position of the interventricular sulcus indicated by the white wire. Same size as figure 6.

posterior through sternum and vertebral column, we shifted the camera so that the optical axis was perpendicular to a tangent which touched the chest at about the anterior axillary line (fig 7). We did this on the assumption that the heart had been dislocated and were desirous of treating it as if it were in the normal position. Having created conditions which simulated the normal, we rotated the heart on its own long axis. The position which the interventricular sulcus takes

before rotation of the heart is best seen in the photograph (fig 7) Rotation of the heart within limits which might be expected to occur clinically did not alter materially the position of the interventricular sulcus In drawing aside the left lung in order to photograph the heart from the side we were duplicating the condition existing in this patient, in whom there was no lung tissue in this region

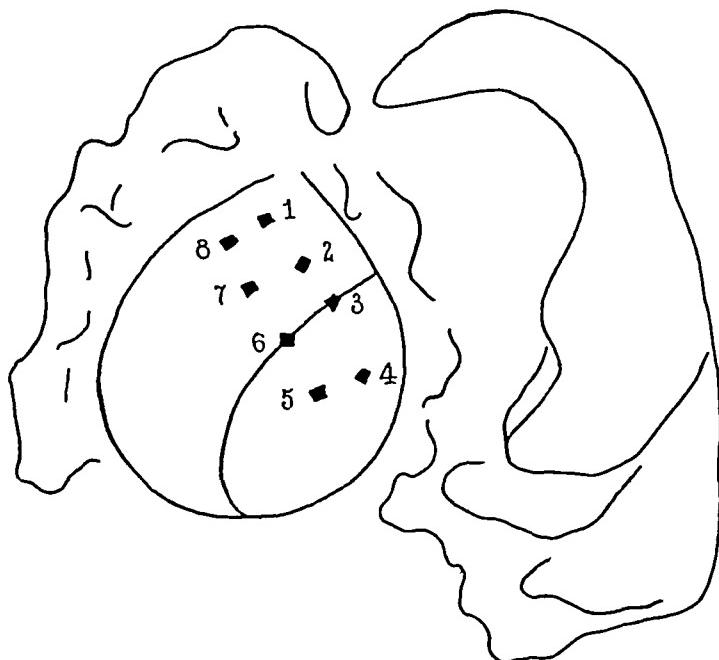


FIG 8 In this figure is shown a drawing made by combining figures 1 and 7 in the following manner A tracing of the heart outline is made from figure 7, on which is indicated the position of the interventricular sulcus A tracing was also made from the photograph of the chest in figure 1 (before reduction) to give the positions of points nos 1 to 8 with reference to one another This tracing was then superimposed on the tracing of the heart in the manner seen in this figure The interventricular groove passes through points nos 3 and 6 Same reduction as in figures 6 and 7

Having established in a photograph the position of the interventricular groove in this anatomical specimen (fig 7), we attempted to see what positions points nos 1 to 8 indicated on the chest wall would take with reference to it The heart outline showing the

position of the interventricular groove was traced on paper. A similar tracing was made of points nos 1 and 8 from the photograph of the chest (fig 1). The second tracing was then superimposed on the first (fig 8). Points nos 1, 2, 7 and 8 lie on the right ventricle, points nos 4 and 5 on the left ventricle, and points nos 3 and 6 in the neighborhood of the interventricular groove (fig 8). Since the points nos 1, 2, 7 and 8 lay on the right ventricle and yielded upward deflections in Lead III, ventricular premature contractions having this form are right ventricular in origin, similarly ventricular premature contractions having downward deflections in Lead III are left ventricular in origin. We are led then to a conclusion based on a right-left ventricular origin of the premature contractions. This, it may be recalled, is the generally accepted view. This conclusion which can be derived from this method of procedure is however not justified for the position of the interventricular groove may have been different in this patient's heart from that in the normal specimen. There is however a strong probability that his heart was in the normal position, for it was practically fixed in the chest as is indicated by the small shift of the electrical axes on changing the patient's position (Dieuaide (17)). Nor did the position of the heart alter sufficiently lying on his right and then on his left side to change the form of the premature contractions derived from points nos 1 to 8.

The view that ventricular premature contractions are left ventricular in origin in which the main deflections are directed upward in Lead III and the reverse, right, can not be maintained in view of our data. There can be little doubt that point no. 8 was over the right ventricle near the base and point no. 4 over the left near the apex. The form of the ventricular premature contractions which we obtained from these points makes such an interpretation impossible. This view is confirmed by the interpretation of an electrocardiogram from a patient which Professor Einthoven has kindly allowed us to use<sup>5</sup> (fig 4). In this patient the greater part of the sternum and ribs was removed by an operation following an accident. Professor Einthoven was able to induce ventricular premature contractions by mechanical stimulation of an area which was over the *right* ventricle. The form of the

<sup>5</sup> We wish to thank Professor Einthoven for his kindness in placing this electrocardiogram at our disposal.

ventricular complexes which he obtained was essentially the same as that of the ventricular premature contractions which we elicited from point no. 8 in our patient (fig. 1)

The significance of the experiments of Rothberger and Winterberg in this connection should be reviewed. It is possible, although improbable, that we repeated in the human subject that part of their experiment in the dog in which they stimulated a series of points on the *left* ventricle in a line from base to apex. On stimulating the base, they obtained ventricular premature contractions in which the first main deflection was inverted in Lead I and upright in Lead III; on stimulating the apex, they obtained ventricular premature contractions in which the first main deflection was inverted in both leads, and on stimulating an intermediate zone, they obtained premature contractions transitional in type. The fact that our curves resemble in direction the three types yielded by the left ventricle of the dog, makes it necessary to consider whether our case and their experiments are analogous, that is to say, whether we also stimulated points on the left ventricle only, and that stimulation of the base of the human left ventricle yields ventricular complexes in which the first main deflection is also upright in Lead III. Against this interpretation is the fact that in Einthoven's patient stimulation of the right ventricle yielded ventricular premature contractions identical with ours in which the first main deflection is upright in Lead III (fig. 4). The question remains undecided, however, whether both left and right ventricles yield similar complexes at their bases, although it is improbable that they should do this.

From the data which we have accumulated, we can not conclude that the base-apex interpretation is correct, neither can we conclude that it is incorrect. When we stimulate a region of the heart which is right ventricle we obtain in the electrocardiogram complexes which are generally identified as being right ventricular in origin (VPCR), but this region undoubtedly is also the base. When we stimulate an area which is left ventricle we obtain ventricular premature contractions which have the form generally accepted as being derived from the left ventricle (VPCL), and this area undoubtedly is also the apex. In a region between these two areas and in a direction running almost transversely, ventricular premature contractions of a transitional

type are obtained. If it were not for the light which we have been able to throw on the location of the interventricular groove, we should be in a dilemma. If we are correct in assigning its location in relation to the points of stimulation we have no choice except to adhere to the two-ventricle interpretation of this problem. We deal with an obvious correlation of anatomical with the electrocardiographic facts. A doubt exists only in the justice of our inference from the similarity of the facts in this case and in the anatomical specimen. But until the reasons for doubt increase or become intensified, there is no adequate ground for turning to the apex base theory. It seems best therefore to continue to designate ventricular premature contractions when the main deflections in Lead III are erect as VPCR, and VPCL when in Lead III they are inverted.

#### SUMMARY AND CONCLUSIONS

From a patient in whom it was possible to stimulate the ventricles of the heart mechanically we have obtained electrocardiograms of artificially induced ventricular premature contractions. The premature contractions fall into three groups on the basis of their form: those having upward deflections in Lead III, those having downward deflections in Lead III, and those having diphasic deflections in Lead III. Although it is not possible to identify which portions of the ventricles lay beneath all of the points stimulated, we are nevertheless reasonably certain that when a point definitely over the right ventricle was stimulated the spiked deflections in the electrocardiogram of the premature contractions were upright in Lead III (VPCR) and inverted when the point stimulated was over the left ventricle (VPCL). To retain the terminology which is in current use seems therefore justified. To consider ventricular premature contractions the main deflections of which are upright in Lead III as originating in the right ventricle, and those the first main deflections of which are inverted in Lead III as originating in the left ventricle, is also justified.

#### BIBLIOGRAPHY

- 1 Einthoven, W. Arch f d ges Phys., 1908, cxxii, 579. Weiteres ueber das Elektrokardiogramm.

- 2 Rothberger, C J, and Winterberg, H Arch f d ges Phys , 1913, chv, 571 Studien ueber die Bestimmung des Ausgangspunktes ventrikularer Extrasystolen mit Hilfe des Elektrokardiogramms
- 3 Einthoven, W Arch internal de phys , 1906, iv, 132 Le Télécardiogramme
- 4 Kraus, F, and Nicolai, G F Berl klin Woch , 1907, xliv, 765 and 811 Ueber das Elektrokardiogramm unter normalen und pathologischen Verhaltnissen
- 5 Nicolai, G F, and Rehfisch, E Zentralb f Phys , 1908, xxii, 57 Ueber das Elektrokardiogramm des Hundeherzens bei Reizung des linken und rechten Ventrikels
- 6 Kraus, F, and Nicolai, G F Das Elektrokardiogramm des gesunden und kranken Menschen Leipzig, 1910
- 7 Nicolai, G F In Nagel, W, Handb d Phys , Braunschweig, 1909, i, 822 Versuche die Richtung der Erregungswelle zu bestimmen
- 8 Kahn, R H Zentralb f Phvs , 1909, xxiii, 444 Ueber das Flektrokardiogramm künstlich ausgelöster Herzkammerschläge
- 9 Kahn, R H Zentralb f Phys , 1910-11, xxiv, 728 Ueber anomale Herzkammerelektrogramme
- 10 Rothberger, C J, and Winterberg, H Zentralb, f Phys , 1910-11, xxiv, 959 Zur Kenntnis des Elektrogrammes der ventrikulären Extrasystolen
- 11 Lewis, Thomas Phil Trans Royal Soc , 1916, B , ccvi, 278 The Spread of the Excitatory Process in the Vertebrate Heart
- 12 Lewis, Thomas The Mechanism and Graphic Registration of the Heart Beat 3rd Ed , London, 1925, 218
- 13 Hoffmann, A Med Kln , 1913, ix, 2025 Ueber künstliche Auslösung von Arrhythmien am gesunden menschlichen Herzen
- 14 Hoffmann, A Die Elektrographie Wiesbaden, 1914, 121
- 15 Cohn, A E Heart, 1922, ix, 311 An Investigation of the Relation of the Position of the Heart to the Electrocardiogram
- 16 Münn, Hubert Arch Int Med , 1920, xxv, 283 A Method of Analyzing the Electrocardiogram
- 17 Dieuaide, F R Arch Int Med , 1925, xxxv, 362 The Electrocardiogram as an Aid in the Diagnosis of Adhesive Pericardial Mediastinitis

## VARIATIONS IN BLOOD FLOW WITH CHANGES IN POSITION IN NORMAL AND PATHOLOGIC SUBJECTS<sup>1</sup>

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The work reported in this paper was undertaken with the following objects in view (a) Correlation of the various factors entering into the physiology of circulation under varying conditions, (b) comparison of the circulatory responses of certain pathologic individuals with those obtained in normal subjects.

The work includes simultaneous measurements of blood flow, rate of ventilation, metabolic rate, pulse rate, pulse pressure, cardiac out-put per systole, vital capacity, tension of carbon dioxide in alveolar air, and tension of carbon dioxide in arterial and mixed venous blood with changes of position. Observations have already been made on most, if not all, of these factors individually for the normal subject, but there have been no reports in which simultaneous observations on all of these several factors have been made. The first work on variations in blood flow caused by changes of posture seems to have been done by Lindhard (1) in 1913. No further data on this subject were collected until Field and Bock (2) in 1925 reported a series of observations on blood flow in normal subjects in three positions in which they showed that there was a diminution of blood flow in the sitting or standing posture as compared with the recumbent position.

The variations in ventilation with changes in position were clearly noted by Liljestrand (3) in 1913.

Changes in metabolic rate with shifts in position have already been

<sup>1</sup> The expenses of this research were defrayed in part by the Tutorial Fund of Harvard University.

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noted by Benedict and Benedict (4) in 1924 and more recently by Turner (5)

An excellent report of the changes in blood pressure, pulse pressure and pulse rate is that of Erlanger and Hooker (6). These observers state in their conclusions "When the standing posture is assumed from the recumbent or the sitting posture, the blood pressures may either rise or fall, the result probably depending largely upon attendant circumstances, such as external temperature, activity of the digestive organs, etc., but the pulse pressure is always diminished and the pulse rate increased." Further work was done on this subject by Sewall (7), who concluded from a study of several hundred cases that there was always a fall in pulse pressure in changing from the recumbent to the standing posture.

The effects of posture on the vital capacity have been known since the time of Hutchinson (8) in the middle of the last century. This observer noted that the vital capacity increased when the subject changed from a recumbent to a sitting or standing position. He also noted that the results obtained in the standing position were greater than those in the sitting posture. Subsequently Bohr (9) in 1907, Christie and Beams (10) in 1922 and 1923 (11) and Rabinowitch (12) in 1923 have confirmed his findings.

The fall in the tension of carbon dioxide in alveolar air has been noted and described by Higgins (13) who found the tension progressively diminished in changing from the recumbent to the sitting and from the sitting to the standing positions, respectively. This, along with a drop in the tension of carbon dioxide in mixed venous blood and an increase in difference between arterial and venous carbon dioxide tension, has for some time been observed by Bock and his co-workers.

Turner (5) has further confirmed these findings.

#### METHOD

All of the observations in this series were made on subjects who had had no breakfast and who had been lying flat in bed with two small pillows for twenty to thirty minutes. The initial observations were made in all cases with the subject in the recumbent posture,

usually in the following order ventilation rate on a calibrated spirometer, circulation experiment proper (i.e., tension of carbon dioxide in alveolar air and tension of carbon dioxide in mixed venous air samples), blood pressure with the arm in the horizontal position, and finally the vital capacity. The subject then sat in a straight backed chair and the previous observations were repeated. Finally the standing posture was assumed and a third series of observations was made. The patient with exophthalmic goitre was so sick that we were forced to allow him to support himself partially by resting his hands on the back of a chair. With this exception, all of the subjects stood quietly without any support.

On one subject (Miss S P) we made a fourth set of observations, viz., in the prone position following the standing observations and after a rest period, the experiment was repeated in the recumbent posture. The agreement with the first set of observations was well within the limits of experimental error. Consequently it did not seem to us that the order in which these sets of observations were made was of any material importance.

We have followed essentially the same details in blood flow determinations as those described by Field and Bock in their first paper on this subject. In brief, this consisted in obtaining four alveolar air samples at the end of expiration and four mixed venous air samples simultaneously, the subject being allowed to breathe in and out of the bag (which contained approximately 6 per cent of carbon dioxide and 94 per cent of oxygen) for periods of 10 to 15 seconds. Following or preceding this, the rate of ventilation was measured by means of a calibrated spirometer. In a few instances we did not change the air in the bag for each set of observations and in these cases we found the results to agree with those in which the air was changed for each position. However, we feel that the latter method is less open to error.

#### RESULTS OF EXPERIMENTS

Tables 1 and 2 may be considered together, as the latter summarizes the important data of the former. The striking finding is the lack of agreement between the metabolic rate and the blood flow, the former showing an increase in every instance but one, the latter a decrease in all but two with the assumption of the sitting or standing

TABLE I  
Blood flow experiments on normal subjects

Experimenter number	Subject, age, weight and height	Position	CO <sub>2</sub> uptake min	RQ	Alveolar CO <sub>2</sub> tension mm	Mixed venous CO <sub>2</sub> tension mm	$\Delta^*$	O <sub>2</sub> capacity ml	Blood flow per minute liters	Venulation per minute liters	O <sub>2</sub> per minute liters	Output per systole cc	Pulse utilization	Pulse rate min	Vital capacity cc		
									ml	mm	ml	cc					
I	Mr J S L 29 years 62 kgm, 169 cm	Lying	189	0.848	40.70	45.84	5	14.21	54	8.92	5.48	223	143	12.2	61	31	3,375
		Sitting	176	0.762	38.76	44.85	6	0.9		6.48	5.44	231	96	17.4	70	24	3,500
		Standing	194	0.788	34.76	42.30	7	5.4		5.54	7.18	247	67	21.8	88	13	3,450
II	Mr R E 26 years 69.9 kgm, 176 cm	Lying	167	0.786	40.93	46.94	6	0.121	52	6.74	5.05	212	121	15.5	58	31	5,200
		Sitting	188	0.704	39.27	47.44	8	1.6		5.36	6.10	267	84	24.4	62	20	5,475
		Standing	216	0.768	32.69	42.13	9	4.44		4.81	7.43	282	56	28.6	88	10	5,600
III	Mr S D B 27 years 85.5 kgm, 181 cm	Lying	245	0.896	39.28	47.83	8	5.52	76	6.80	6.82	273	97	20.4	70	37	4,125
		Sitting	285	0.850	38.02	47.18	9	1.5		7.06	8.36	335	105	24.1	67	29	4,750
		Standing	307	0.887	33.38	45.08	11	7.0		5.59	9.26	344	70	31.4	80	23	4,925
IV	Mr S A O 27 years 77 kgm, 172.5 cm	Lying	189	0.783	40.70	46.70	6	0.022	52	7.64	5.00	241	121	14.8	63	32	4,150
		Sitting	218	0.804	38.48	46.40	7	9.2		6.19	6.26	271	109	20.5	57	28	4,475
		Standing	232	0.795	30.28	42.05	11	7.77		4.01	7.40	292	54	34.0	74	18	4,600
V	Miss V. J P 20 years 64.5 kgm, 170 cm	Lying	281	1.180	26.51	36.00	9	50.19	19	5.82	9.20	234	90	22.4	65	30	2,525
		Sitting	262	1.035	23.99	34.28	10	29		4.64	8.80	253	58	29.9	80	20	2,700
		Standing	260	0.993	21.48	34.23	12	7.75		3.55	10.20	262	36	40.5	98	11	2,700

VI	Miss S P 21 years 56 kgm, 163.5 cm	Lying	138	0 742	36 50	43 42	6 92	18 05	4 30	5 38	186	56	25 2	77	29	2 400
		Sitting	166	0 699	37 60	44 12	6 52	5 53	6 68	237	67	25 0	82	32	2 675	
		Standing	173	0 884	32 89	41 90	9 01	4 06	7 12	196	41	28 1	100	15	2 750	
VII	Miss H N L 23 years 66.5 kgm, 173.5 cm	Lying	152	0 810	33 90	42 43	8 53	3 79	6 15	188	54	28 8	70	30	2 350	
		Sitting	193	0 752	37 33	44 83	7 50	5 64	5 42	257	83	24 7	68	30	4 150	
		Standing	189	0 772	31 27	41 37	10 10	3 88	5 54	245	42	34 2	92	18	4 250	
VIII	Mr L M H. 29 years 80 kgm, 185 cm	Lying	227	0 992	39 43	47 68	8 25	21 22	6 43	6 38	246	102	17 7	63	27	5 200
		Sitting	227	0 772	37 46	45 63	8 17	6 17	7 20	294	101	23 7	61	22	5 450	
		Standing	192	0 808	35 36	42 98	7 63	5 39	6 43	238	62	21 9	87	12	5 575	
IX	Mr C D 38 years 172 cm.	Lying	185	0 866	43 15	49 12	5 97	20 22	8 12	5 08	211	176	13 7	46	27	5 000
		Sitting	229	0 891	38 56	47 00	8 43	6 21	6 84	257	135	21 5	46	28	5 250	
		Standing	216	0 854	35 85	45 57	9 72	4 85	7 40	253	87	27 1	56	17	5 400	
X	Mr T D J 27 years 55 kgm, 161 cm	Lying	162	0 871	39 27	46 77	7 49	22 64	5 23	5 21	186	75	17 4	70	32	2 875
		Sitting	170	0 814	38 56	47 73	9 17	4 27	6 85	209	56	22 7	76	30	2 975	
		Standing	171	0 802	33 91	46 16	12 25	3 02	7 39	213	34	32 9	89	23	3100	

\*Δ = difference between alveolar and mixed venous carbon dioxide tensions.

TABLE 2  
Percentile variation from supine position in normal subjects

Experiment number	Subject	Position	$\text{CO}_2$ expired	Alveolar $\text{CO}_2$ tension	Mixed venous $\text{CO}_2$ tension	$\Delta^*$	Blood flow	Ventilation	Metabolic rate	Pulse rate	Pulse pressure	Vital capacity	Output per systole
I	Mr J S L	Sitting	-6.9	-4.8	-2.2	+18.7	-27.4	-0.7	+3.6	+14.8	-22.6	+3.7	-32.9
II	Mr R E	Standing	+2.6	-14.6	-7.7	+47.2	-37.9	+31.0	+10.8	+44.3	-58.1	+2.2	-53.1
		Sitting	+12.6	-4.1	+1.1	+35.8	-20.5	+20.8	+25.3	+6.9	-35.5	+5.3	-30.6
III	Mr S D B	Standing	+29.3	-20.1	-10.2	+57.1	-28.6	+47.1	+32.4	+51.8	-67.8	+7.7	-53.7
		Sitting	+16.3	-3.2	-1.4	+7.0	+3.8	+22.6	+22.7	-4.3	-21.6	+15.2	+8.3
IV	Mr S A O	Standing	+25.3	-15.0	-57.5	+36.8	-17.8	+35.8	+26.0	+14.3	-37.9	+19.4	-27.8
		Sitting	+15.3	-5.5	-0.6	+32.0	-17.0	+25.2	+12.4	-9.5	-12.5	+7.8	-9.9
V	Miss V J P	Standing	+22.7	-22.1	-10.0	+96.2	-47.5	+48.0	+21.2	+17.5	-43.8	+10.8	-55.4
		Sitting	-6.8	-9.5	-4.8	+8.3	-20.3	-4.4	+8.1	+23.1	-33.3	+6.9	-35.6
VI	Miss S P	Standing	-7.5	-19.0	-4.9	+34.2	-39.0	+10.9	+12.0	+50.8	-63.4	+6.9	-60.0
		Sitting	+20.3	+3.0	+1.6	-5.8	+28.6	+24.2	+27.4	+6.5	+10.3	+11.5	+19.6
VII	Miss H M L	Standing	+25.4	-9.9	-3.5	+30.2	-5.6	+32.4	+5.4	+29.9	-48.3	+14.6	-26.8
		Sitting	-9.0	-0.7	+3.3	+28.9	-29.5	-2.2	+9.9	-16.1	-11.8	+15.3	-16.2
VIII	Mr L M H	Standing	-10.9	-16.9	-4.7	+73.6	-51.5	0.0	+4.7	+13.6	-47.1	+18.1	-57.6
		Sitting	0.0	-5.0	-4.3	-1.0	-4.0	+12.9	+19.5	-3.2	-18.5	+4.8	-1.0
		Standing	-15.4	-10.3	-9.9	-7.5	-16.2	+0.8	-3.3	+38.1	-55.6	+7.2	-39.2

$\Delta$	Mr C D	Sitting	+23 8	-10 6	-4 3	+41 2	-23 5	+34 7	+21 8	0 0	+3 7	+5 0	-23 3
		Standing	+16 8	-16 9	-7 2	+62 8	-40 3	+45 7	+19 9	+21 8	-37 1	+8 0	-50 6
$\Delta$	Mr T D J	Sitting	+4 9	-1 8	+2 1	+22 4	-18 4	+31 5	+12 4	+8 6	-6 3	+3 5	-25 3
		Standing	+5 6	-13 6	-1 3	+63 6	-42 3	+41 8	+14 5	+27 2	-28 1	+7 8	-54 7
	Av	Sitting	+7 1	-4 1	-0 95	+18 8	-12 8	+16 4	+16 3	+2 7	-14 8	+7 9	-14 7
	Av	Standing	+9 4	-15 8	-11 6	+49 4	-32 7	+29 4	+14 3	+30 9	-48 7	+10 3	-47 9

\*  $\Delta$  = difference between alveolar and mixed venous carbon dioxide tensions.

TABLE 4  
Percentile variation from supine position in pathologic subjects

Experiment number	Subject	Diagnosis	Position	$\text{CO}_2 \text{ exhaled}$	Mixed venous $\text{CO}_2$ tension	$\Delta^*$	Blood flow	Ventilation	Metabolic rate	Pulse rate	Vital capacity	Output per systole
XI	Mr S I J	Convalescence	Standing	+47.1	-17.2	-8.3	+37.4	-1.5	+43.8	+17.0	+48.7	-75.0
XII	Mr J J F	Convalescence, mitral stenosis, well compensated	Sitting	+15.3	-9.8	-2.3	+47.3	-27.8	+20.9	+25.5	+10.0	-16.7
			Standing	+19.0	-18.0	-8.1	+59.0	-31.9	+31.4	+27.9	+72.5	-50.0
XIII	Mr J McM	Convalescence	Sitting	+8.0	-0.6	+5.2	+70.7	-41.2	+29.9	+3.2	+5.0	+7.5
			Standing	+10.7	+1.6	+11.2	+118.7	-54.2	+24.3	+26.3	+35.0	-17.5
XIV	Mr C FW W	Convalescence	Sitting	+52.7	-11.5	-10.1	-5.8	+51.8	+74.2	+34.5	+30.0	-35.0
			Standing	+36.3	-17.8	-13.8	-2.0	+25.6	+87.2	+7.5	+83.3	-60.0
Av	Sitting			+25.3	-7.3	-2.6	+37.4	-5.7	+41.7	-	-	-
	Av Standing			+28.3	-12.9	-4.8	+53.3	-15.5	+46.7	+19.6	+59.9	-50.6
XV	Mrs M J J	Mitral stenosis, aortic regurgita tion well compensated	Sitting	+48.4	-6.8	+1.0	+40.2	+3.6	+82.6	+27.0	0.0	+6.5
			Standing	+12.4	-6.9	+2.2	+48.3	-22.7	+36.3	+3.5	+15.7	+29.0
XVI	Mr J C V	Toxic goiter	Standing	+21.9	-3.2	+3.7	+29.0	+17.9	+53.5	+41.6	+15.4	-37.9
												+5.2
												+2.1

XVII	Mrs E M D	Myxedema	Sitting	+13 2	+21 2	+16 0	-5 9	+24 8	+27 4	+14 5	+20 7	-53 6	+12 6	+3 1
			Standing	+9 3	+11 9	+7 8	-9 5	+26 1	+30 9	+15 2	+37 9	-42 9	+18 4	-9 2
XVIII	Mrs J W	Myxedema	Sitting	+32 3	-13 7	-9 3	+2 1	+21 4	+46 5	+46 5	0 0	+11 1	+11 5	+19 7
			Standing	+27 1	-23 0	-14 6	+7 4	-9 6	+55 9	+66 2	+15 6	0 0	+4 2	-23 2
XIX	Mrs M V L	Myxedema	Sitting	+14 7	+0 6	-2 0	-11 2	+29 9	+31 4	+24 6	+9 7	-30 8	+1 0	+20 4
			Standing	+22 1	-1 6	-2 9	-7 5	+32 5	+42 0	+26 8	+12 9	-50 0	-2 1	+6 1
			A <sub>1</sub>	+20 2	+2 7	+1 6	-5 0	+25 4	+35 8	+28 5	+10 1	-24 4	+8 4	+14 4
			A <sub>2</sub>	+19 5	-4 2	-3 2	-3 2	+16 3	+42 9	+36 1	+22 1	-31 0	+6 8	-8 8

\*  $\Delta$  = difference between alveolar and mixed venous carbon dioxide tensions.

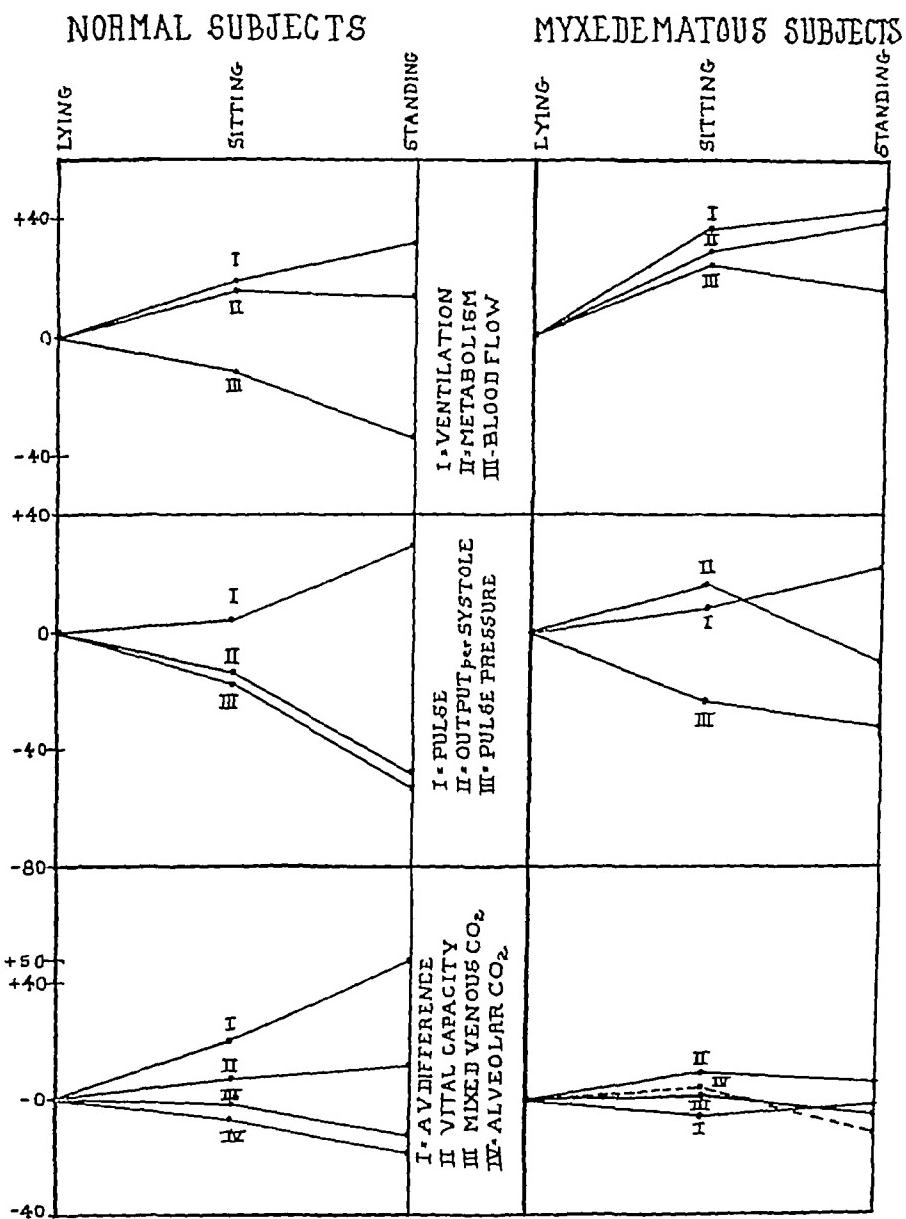


FIG 1

servations on vital capacity as related to posture in patients with diminished vital capacity due to different causes. While it is true that they found a greater increase with change of posture in such patients with orthopnea, yet they found no increase above normal in such patients without orthopnea. Our subjects showed no diminution in vital capacity and no orthopnea.

The only other subject with a cardiac lesion in this series is J J F who had mitral stenosis without signs of heart failure. He did not present findings similar to the above.

This series includes only one subject with exophthalmic goiter. He showed in the standing posture an increase in blood flow which was not nearly commensurate with the increase in metabolism. (The subject was so toxic that it is difficult to rely on the findings in his case.)

The short series of cases with myxedema presented the greatest variations from the normal. It is significant that the blood flow instead of being diminished on changing from the lying to the sitting or standing positions was increased. Although it is true that here we were dealing with very much smaller values as regards blood flow than in the normal subjects, yet the percentile differences were so consistently increased as to seem of some importance. The tensions of carbon dioxide in alveolar air were low. The unusually low values in J W were, no doubt, due to hyperventilation. The explanation of the other low values is difficult.

Table 5 shows the average coefficient of utilization of oxygen in the three positions in the various types of subjects studied. It will be seen that there was a definite increase in the coefficient of utilization of oxygen in changes from the lying to the sitting and standing postures. The subjects with heart lesions, exophthalmic goiter and myxedema presented the highest values.

In figure 1 are shown graphically what happens to the various factors discussed as a result of posture, (a) in the average normal, and (b) in subjects with myxedema. If such a short series can be taken as presenting trustworthy evidence, there is seen to be a very striking difference in the subjects with myxedema.



# A COMPARATIVE STUDY OF THE ROTATORY AND REDUCING PROPERTIES OF ULTRAFILTRATES FROM BLOOD PLASMA

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Attempts to measure and define the nature of blood sugar present certain difficulties largely because the more familiar methods at our disposal are dependent upon certain properties which substances other than glucose may also exhibit. Reducing power, optical activity and the property of being fermented, notably by yeast, represent the most important of these properties. The well known reducing methods employed for blood sugar estimation are apparently sufficiently accurate for general clinical purposes, but it is recognized that there may be small amounts of reducing substances in the blood which cannot be attributed to glucose. The exact nature of these has not been accurately defined, although it is known that uric acid, creatinine, creatine, glucuronic acid, pentoses, disaccharides, purines and adrenalin have the power of reducing the reagents commonly used in blood sugar determinations. Hiller, Linder and Van Slyke (1) have recently studied the other reducing substances in blood and in a series of reducing determinations both before and after the removal of the glucose by yeast fermentation and by the glycolytic action of blood they found that the residue of reducing substance was equivalent to 0.01 to 0.03 per cent of glucose.

The use of the polariscope for the estimation of blood sugar may be subjected to similar criticism, owing to the presence of other optically active substances, although this fact does not seem to have been emphasized in recent work on the specific rotation of the glucose of normal blood (12) (19). The literature covering the general subject

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of the rotatory properties of blood is by no means as voluminous as that covering its reducing properties but many comparative polariscopic and reducing studies have been made and the results have seemed to be of sufficient importance to warrant a brief review.

In 1901 Paul Mayer (2) in a comparative study of the rotatory and reducing values of filtrates of animal and human blood, noted that the discrepancies between the two were appreciable. He attributed this difference largely to the presence of levorotatory substances which he succeeded in demonstrating after removing the sugar by fermentation and concluded that glucuronic acid, probably present in the conjugated state, was responsible for this levorotation and represented a normal constituent of the circulating blood.

These findings were in large measure confirmed and also amplified by the work of Lepine and Boulud (3), who in a series of studies on animal blood, found that conjugated glucuronic acid was always present and that it was increased in amount if the blood was allowed to stand, particularly if the glycolytic action of whole blood was not prevented. These investigators suspected that the relation between glucose and glucuronic acid formation was intimately associated with the problem of glucose metabolism.

In 1908 Michaelis and Rona (4) dialyzed blood plasma against a series of glucose solutions of varied concentration of which some were approximately isotonic with the estimated glucose content of blood. The dialysates were studied by polarimetric and reducing methods. It is somewhat difficult to interpret their results as to agreement between rotatory and reducing power of the actual blood sugar, but in general, fairly close agreement between the rotatory and reducing values of the dialysates was noted. In a later study, however, on protein-free blood filtrates Rona and Takahashi (5) compared the rotatory value of the filtrates before and after fermentation and noted the occasional presence of traces of levorotatory substances.

Similarly Oppler (6), working with blood filtrates in which the protein had been precipitated with phosphotungstic acid, made a series of comparative studies on blood sugar by reducing, polariscopic and fermentation methods. He obtained fairly close agreement between the rotatory and reducing values of these filtrates but found in some instances (about 40 per cent) that the rotatory values were lower than the reducing values. He attributed these discrepancies to the presence of levorotatory substances and although he did not define these substances he suggested that they were probably acid in nature, and probably analogous to those found in urine. In concluding he stated that these substances should be considered as sources of error in any method which attempts to estimate blood sugar by the use of the polariscope.

In a later study on the rotatory value of protein free blood filtrates, Lyttkens and Sandgren (7), noted that the presence of other optically active substances in these filtrates distinctly interferes with the accuracy of this method for the estimation of blood sugar.

On the other hand Griesbach and Strassner (8) obtained fairly close agreement in their comparative study of the rotatory and reducing values of blood filtrates and concluded that the polariscopic method was a practical one for the estimation of blood sugar. Fairly close agreement between rotatory and reducing values was also reported by Maase and Tachau (9).

In a more recent and rather comprehensive study Stepp (10, 11) has discussed the variables which enter into the problem of blood sugar estimation. He has placed particular emphasis upon the error in the reducing methods brought about by the presence of other reducing substances such as uric acid, creatinine, creatine, amino acids, etc., as well as similar errors in rotatory determinations caused by the presence of  $\beta$ -oxybutyric acid, amino acids and glucuronic acids, particularly the latter. In his experimental data on blood filtrates, fairly close agreement between rotatory and fermentation values were obtained, but as a rule the rotatory values proved to be lower than the reducing values by about 20 to 30 per cent. He also noted that in the course of concentrating the blood filtrates there was a fall in the initial reducing values so that they approached the rotatory ones and from these observations he concluded that certain volatile reducing substances might be present. His observations were extended to include studies on the blood of nephritics and diabetics and he reported that in diabetes the higher the blood sugar the greater was the difference between reduction and rotation.

A somewhat different interpretation of the differences between rotatory and reducing values in the blood of normal individuals and in diabetics was introduced in 1923 by the work of Winter and Smith (12). These investigators precipitated the protein from samples of human and animal blood by the method of Folin and Wu. The resulting filtrate, after concentration by vacuum distillation at 40°C was then reprecipitated with alcohol in order to remove existing traces of protein and reconcentrated a process which under favorable circumstances required about 6 hours. On comparing the rotatory value of the final protein free extract with the reducing value they found that in normal individuals the initial rotatory values were well below those of the reducing values but rose on standing until in the course of a few days they were practically equal to the reducing values. They concluded that these low rotatory values might indicate the presence in normal blood of a type of glucose which was not the stable  $\alpha, \beta$ -glucose, but a less stable variety such as that identified by Irvine and his coworkers and styled  $\gamma$ -glucose. They further suggested that the transformation of  $\alpha, \beta$ -glucose into  $\gamma$ -glucose might represent an important preliminary step in the preparation of glucose for utilization by the body.

Considerable doubt has been cast upon the conclusions drawn by Winter and Smith by Hewit (13), and by Eadie (14). The latter repeated the work and obtained not only polarimetric readings similar to those reported by Winter and Smith for normal blood but also others of an exactly opposite character. Eadie is quoted by Macleod (15) as having shown that in extracts of normal blood polarimetric readings are often obtained which are less dextrorotatory than they should

be, as judged by the reducing power, and which slowly become greater on standing but that this, instead of indicating an increase in the specific rotation of the glucose present, may depend upon the presence of glucosides which gradually become hydrolyzed on standing, or of traces of other levorotatory substances which are gradually destroyed.

Van Creveld (16) also repeated in some measure Winter and Smith's work, but eventually abandoned the lengthy methods of deproteinization of blood as advocated by Winter and Smith, choosing instead to work with the aqueous humor of the eye, serum ultra-filtrates and artificially produced transudates. With the aqueous humor he noted that reduction and optical rotation determinations showed close agreement and mutarotation could not be detected. With serum ultra-filtrates and transudates no changes were noted in rotation, but there was always a small difference between rotation and reduction with the reducing values slightly less than the rotatory ones.

Later Denis and Hume (17) repeated the experiments of Winter and Smith and found that the percentage of glucose in the blood extracts was invariably higher when determined by reduction than when calculated from rotation. They also attributed this to the presence in the blood, of substances other than glucose which were responsible for a part of the optical rotation and of the reducing power of deproteinized extracts. In fact, similar discrepancies between rotatory and reducing values were observed by these investigators with "synthetic" blood extracts prepared from glucose, salts and nitrogenous extractives which were subjected to a distillation and extraction process exactly similar to the one used on blood.

Quite recently Visscher (18) has reported an interesting study on the rotatory properties of a series of human and animal blood filtrates including a repetition of the procedure followed by Winter and Smith. He found that the initial rotatory values of blood filtrates in which protein precipitation had been carried out by the Folin and Wu method were below the reducing values and in one instance an initial levorotatory value was observed. On standing this rotatory value rose somewhat irregularly in the course of a few days, to be followed by a rapid fall. By using different protein precipitants he obtained somewhat different initial rotatory values and on standing different fluctuations. His experiments did not suggest that the findings were due to the presence of a relatively unstable form of glucose which gradually reverted to the more stable form, but rather that substances other than glucose, were influencing these values.

However, Lundsgaard and Holbøll (19) have again raised the question as to whether the discrepancies between rotatory and reducing values in the blood of normal individuals may not indicate changes in specific rotation of the glucose present. They employed a different method in order to obtain a protein free extract of blood which would be suitable for polaroscopic study. This consisted of dialyzing samples of blood, to which 2 per cent sodium fluoride had been added to prevent coagulation and glycolysis, against 0.9 per cent solutions of NaCl for a

period of 1½ hours and studying the dialysates thus obtained. These investigators noted that in normal individuals the rotatory values expressed in terms of glucose, were considerably lower than the reducing values if the determination was made on the same day that the blood sample was obtained. At the end of 48 hours, however, the rotatory values were found to have risen until they were practically in agreement with the reducing values. In diabetic individuals, on the other hand the rotatory and reducing values were found to show remarkably close immediate agreement. On the basis of these findings they suggested that the blood sugar of normal individuals might have a lower specific rotation than that of glucose although it eventually reverted to  $\alpha\beta$  glucose on standing. This interpretation is in accord with the "new glucose" theory that these authors have previously formulated (20), which suggests that insulin together with other substances may have a direct action upon the sugar molecule in preparing it for utilization by the body.

Since submitting this article for publication my attention has been called to a recent paper by Anderson and Carruthers (26) on the relation between optical activity and reducing power of normal blood filtrates in which the views proposed by Lundsgaard and Holbøll have been critically reviewed. These investigators employing concentrated ultrafiltrates, dialysates and alcohol extracts of plasma observed that the rotatory power of these solutions was low as compared to its reducing power. They did not note any fluctuations of the rotatory values on standing but they did note that reversible changes in these rotatory values were brought about by altering the pH of the solutions. It was suggested that these results were due to the presence in plasma of one or more optically active substances other than glucose. Laked corpuscles were shown by these authors to yield strongly levorotatory ultrafiltrates which have little reducing power.

The review of the literature given above may be summarized by the statement that the majority of investigators who have studied the rotatory values of blood have found somewhat irregular and inconstant differences between these values and the reducing values and most investigators have attributed these differences to the presence of optically active and reducing substances other than glucose. A number of rather divergent views have, however, been expressed with regard to the significance and relationship which these differences may have to the question of glucose metabolism, notably the early views of Lepine, who attributed these differences largely to the presence of glucuronic acid and who emphasized the importance which conjugated glucuronic acid might play as a product of glucose metabolism. Again we have the significance of these differences in rotatory and reducing values viewed from another angle in which they are not

attributed to the actual presence of optically active substances other than glucose but rather to the possibility that the specific rotation of the glucose in normal blood may be lower than that of  $\alpha, \beta$ -glucose. It has been suggested that this altered glucose may be a less stable form such as  $\gamma$ -glucose, or Lundsgaard's and Holbøll's "new glucose," and may represent one of the normal stages through which  $\alpha, \beta$ -glucose is prepared for utilization by the body.

Unquestionably, the relationship which the rotatory power has to the actual glucose content of blood and its bearing upon the question of glucose metabolism would seem to warrant a great deal of further study. The series of experiments recorded below deals only with the question from one angle, namely, an attempt to define the limits of the differences between rotatory and reducing values in blood plasma in normal individuals, to record the fluctuation of the rotatory values of a given specimen over a period of several days and finally to study the rotatory power of plasma after the sugar has been removed by the glycolytic action of whole blood. It is not within the scope of this paper to define the nature of the optically active substances, other than glucose or to discuss their relationship to glucose metabolism.

#### METHODS

The measurement of the rotatory value of normal blood offers difficulties owing to the fact that the physiological concentration of optically active substances is small. Hence accuracy is limited, unless the amount of blood filtrate obtained is large, so that a long polariscope tube may be filled. Such large quantities of blood as are required for these determinations are not always easy to procure from human beings. In selecting a method for obtaining a protein free extract of blood which would be suitable for polaroscopic study, the use of collodion membranes as employed by Lundsgaard and Holbøll for the separation of the blood electrolytes from the protein was considered the most satisfactory. The methods of precipitation of the blood protein, filtration and concentration of the filtrate used by other investigators were rejected on account of the length of the process and because of the introduction of variable factors. Instead of using blood dialysates, however, such as were studied by Lundsgaard and Holbøll, ultrafiltrates from the collodion sacs were employed. This method has advantages in that an extract of blood can be obtained in which the resulting glucose concentration in the filtrate more nearly approaches that of the blood plasma. The higher concentration tends to increase the accuracy of the determinations and the dilution factor is eliminated.

The collodion sacs were prepared from a 7 per cent solution of Du Pont's par-

Iodion dissolved in a solution of 75 per cent by volume of ether and 25 per cent of absolute alcohol. In the preliminary experiments on the preparation of suitable collodion membranes the principles emphasized by Eggerth (21) were followed. The standard eventually adopted was that the sacs should be impermeable to hemoglobin but easily permeable to glucose. In making a sac, the inside of a test tube was coated with collodion, drained and allowed to dry for about 4 or 5 minutes until the collodion was quite firm. The tube was then filled to the brim with 70

TABLE I

*Showing the error encountered in 15 reducing and rotary determinations on a series of standard glucose solutions of graded values\**

Calculated values gram per 100 cc	Reducing values (Folin and Wu) gram per 100 cc	Difference between reducing and calculated values gram per 100 cc	Rotatory values gram per 100 cc	Difference between rotatory and calculated value gram per 100 cc	Difference between rotatory and reducing value gram per 100 cc
0.150	0.148	-0.002	0.149	-0.001	0.001
0.140	0.138	-0.002	0.147	+0.007	0.009
0.130	0.131	+0.001	0.126	-0.004	0.005
0.120	0.120	0.000	0.110	-0.010	0.010
0.110	0.109	-0.001	0.124	+0.014	0.015
0.100	0.099	-0.001	0.096	-0.004	0.003
0.090	0.092	+0.002	0.102	+0.012	0.010
0.080	0.080	0.000	0.076	-0.004	0.004
0.070	0.068	-0.002	0.075	+0.005	0.007
0.060	0.057	-0.003	0.068	+0.008	0.009
0.050	0.055	+0.005	0.066	+0.016	0.011
0.040	0.038	-0.002	0.041	+0.001	0.003
0.030	0.026	-0.004	0.035	+0.005	0.009
0.020	0.018	-0.002	0.020	0.000	0.002
0.010			0.008	-0.002	
Max. Dif		0.005		0.016	0.015
Av Dif		0.002		0.007	0.007

\* All of the values are expressed in grams of glucose per 100 cc.

per cent alcohol and allowed to stand for 20 minutes. Distilled water was then substituted for the alcohol for a period of 1 hour. At the end of this time the sac was usually found to have contracted slightly from the tube wall and could be drawn out with relative ease. The sacs were then tested for leaks and impermeability to hemoglobin by filling them with a solution of hemoglobin and subjecting them to a negative pressure of 200 mm. of mercury. The selected ones were kept in water to which a few crystals of thymol had been added and were used repeatedly over a period of about 3 to 4 weeks. The method of ultrafiltration was that described by Marshall and Vickers (22).

Polariscopic readings were made with a Reichert instrument, using a 189 mm tube with a Mazda lamp with a dichromate solution filter as the source of light. The value of  $+52.8^\circ$  was taken for the specific rotation of glucose. In every instance the average of a series of at least 15 readings was determined, together with a series of readings of the zero point.

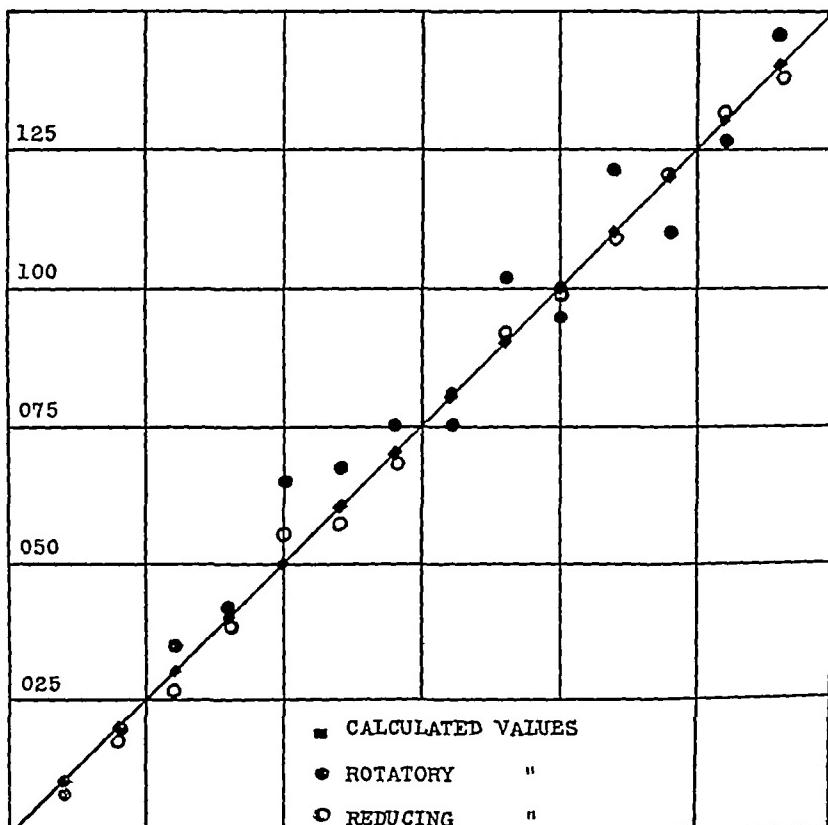


FIG 1 REDUCING AND ROTATORY DETERMINATIONS ON A SERIES OF STANDARD GLUCOSE SOLUTIONS OF GRADED VALUES, RANGING FROM 0.010 TO 0.150 GRAM OF GLUCOSE PER 100 CC

For the reducing determination two methods were employed, that of Folin and Wu (23) and also that of Hagedorn and Jensen (24).

Blood samples of from 60 to 250 cc were collected from normal individuals, from general hospital patients and, in some instances, from cadavers within 10 to 30 minutes after death. The blood was collected aseptically using sodium citrate and sodium fluoride as anticoagulant and antiglycolytic agents. After collection, the specimen was immediately centrifuged and the cells discarded.

The plasma was placed in a collodion sac and subjected to a negative pressure of 150 to 200 mm of mercury for filtration. About 11 or 12 cc. of filtrate were necessary for the determinations, as 10 cc. were required to fill the polariscope tube and at least 1 cc. was used for the initial reducing determination. To procure this amount, from 3 to 5 hours of filtration were generally necessary. Initial rotatory and reducing determinations were done in all instances on the same day on which the specimen was collected. The solution was then left in the polariscope tube which was placed in the ice box for an interval of 2 to 5 days, during which time a series of rotatory readings were made at room temperature. At the end of this time a final reducing determination was made.

For the standardization and the determination of the degree of experimental error which the rotatory and reducing methods employed would show, the following procedure was used. A solution of Merck's pure dextrose was prepared in distilled water and its concentration accurately determined with the polariscope using a 4 dm tube. From this standard a series of 15 graded solutions were prepared by dilution, in which the estimated glucose concentration ranged from 0.150 to 0.010 gram per 100 cc., corresponding to the range of glucose concentration which the blood samples included in this series show. The rotatory and reducing determinations made on this series of solutions are shown in table 1 and figure 1.

The maximum limit of experimental error for both of the reducing methods employed in this experiment had been previously established in a series of determinations on a glucose solution with a concentration of 0.100 gram per 100 cc., as 1.7 per cent, the average error being about 1 per cent. The differences between the reducing values and the calculated values of the solutions in this series fall practically within this range in the solutions with concentrations ranging from 0.070 gram to 0.150 gram per 100 cc. Below this range, i.e., 0.070 to 0.010 gram, the error becomes greater. It will also be noted that the differences between rotatory and reducing values is considerable showing a maximum difference of 0.015 gram of glucose per 100 cc. and an average of 0.007 gram of glucose per 100 cc. This error proved to be the same in actual magnitude for any solution of glucose with a concentration within the range of 0.010 to 1.000 gram per 100 cc. The experimental results of this paper have been viewed critically from the standpoint of this error and all of the rotatory values are considered as having probable deviations, at least to the extent of  $\pm 0.007$  gram of glucose per 100 cc.

#### EXPERIMENTAL

A small series of blood samples obtained from normal individuals was first examined to ascertain the relation of the reducing values of the ultrafiltrates to that of the plasma and to the rotatory values of the ultrafiltrates. The degree of fluctuation which the rotatory values exhibited on standing was also studied. The results on 5 such blood samples are given in table 2.

From table 2 it will be noted that the reducing values of the ultra-filtrates proved to be consistently higher (averaging about 10 per cent)

TABLE 2  
*Reducing and rotatory values encountered in 5 normal individuals\**

Experiment number	Reducing value of plasma	Reducing value of ultra filtrate	Initial rotatory value	24 hour rotatory value	48 hour rotatory value	72 hour rotatory value	96 hour rotatory value	Final reducing value
	gram per 100 cc	gram per 100 cc	gram per 100 cc	gram per 100 cc	gram per 100 cc	gram per 100 cc	gram per 100 cc	gram per 100 cc
37	0.077	0.085	+0.037	+0.051	+0.021			
38	0.099	0.109	+0.009	+0.007	0.000			0.109
40	0.084	0.086	-0.031	-0.003	+0.031	+0.011	+0.009	0.084
41	0.096	0.104	-0.003	-0.028	+0.012	+0.039	+0.049	0.102
42	0.072	0.082	+0.033	+0.013	+0.016	+0.005	+0.013	0.084

\* All of the values are expressed in terms of grams of glucose per 100 cc although the levorotatory values are, of course, arbitrarily represented in such units

TABLE 3  
*Reducing and rotatory values encountered in 13 hospital cases*

Experiment number	Diagnosis	Post mortem collection of blood	Reducing value of ultra-filtrate	Initial rotatory value	24 hour rotatory value	Initial (R P)	24-hour (R P)
			gram per 100 cc	gram per 100 cc	gram per 100 cc		
35	Arteriosclerosis	No	0.092	+0.073	+0.073	0.019	0.019
28	Salvarsan poisoning	No	0.095	+0.063	+0.050	0.032	0.045
26	Lobar pneumonia	No	0.102	+0.094	+0.101	0.008	0.001
33	Lobar pneumonia	Yes	0.071	+0.004		0.067	
1A	Cardiac decompensation	No	0.102	+0.115			
2A	Cerebral hemorrhage	No	0.064	+0.085			
43	Cardiac decompensation	No	0.134	+0.133	+0.105	0.001	0.029
27	Cardiac decompensation	No	0.102	+0.076	+0.103	0.026	
20	Cirrhosis of liver	No	0.075	+0.018	-0.011	0.057	0.086
25	Gun shot wound of head	Yes	0.134	+0.096	+0.086	0.039	0.049
8	Carbon monoxide poisoning	Yes	0.066	+0.027		0.039	
6	Fracture of femur	Yes	0.070	-0.011		0.086	
11	Laceration of liver	Yes	0.076	-0.005		0.071	
Maximum						0.086	0.086
Av						0.032	0.032

than that of the plasma, a difference which may be attributed to the concentration of glucose brought about by the elimination of the

plasma protein. It will also be noted that in every instance the initial rotatory values were considerably below the reducing values of the ultrafiltrates and in one instance the values were levorotatory

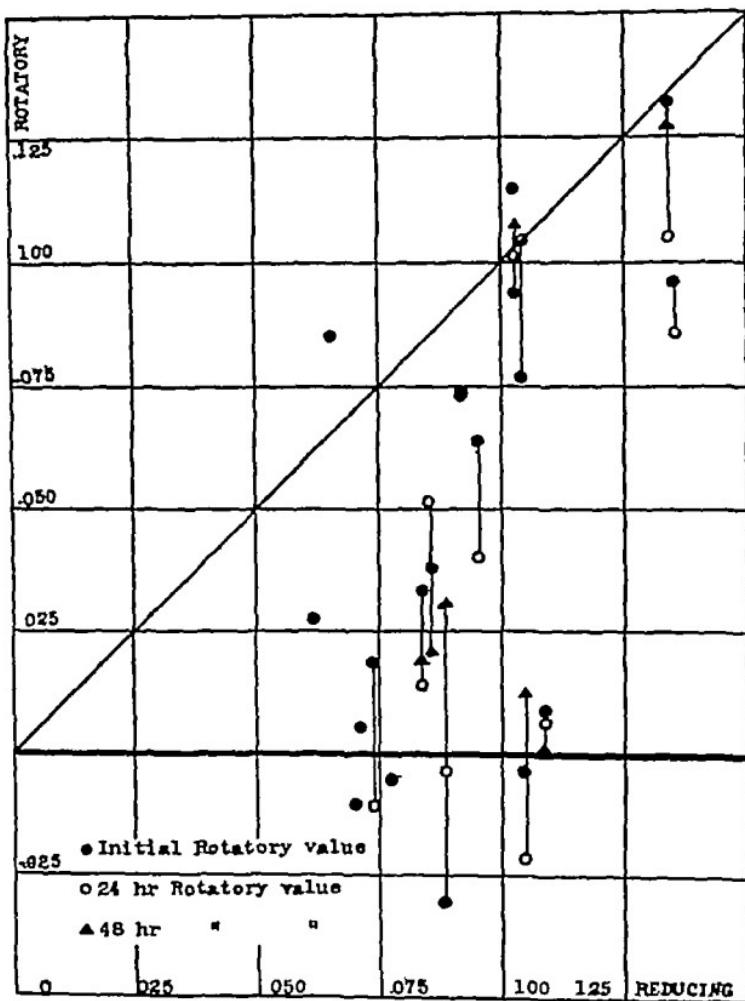


FIG 2 REDUCING AND ROTATORY VALUES ENCOUNTERED IN 18 ULTRAFILTRATES FROM NORMAL INDIVIDUALS AND HOSPITAL PATIENTS  
Daily fluctuations of the rotatory values are charted in 12 instances

be less than the dimin  
diminish with the remo

Initially attempts we  
samples by fermentatio  
satisfactory. The proc  
of relying on the glycol  
sugar. Although this i  
seemed to be more servi

The procedure follo  
the collection of fairly l  
containing crystals of s

*Reducing an*

Experiment number	First	
	Reducing	
	gram per 100 cc	gra 100
2A	0 064	+0
26	0 102	+0
33	0 071	+0
35	0 092	+0
0x*	0 102	+0

\* Defibrinated ox blood

two portions and to th  
was added to prevent  
specimen was then cen  
plasma, of which the  
The second portion w  
or at a temperature of  
of which time rotato  
its plasma ultrafiltrate

The results of a se  
above on four sample  
given in table 4 and  
four experiments gly

perature and, as a result, the reducing power in the ultrafiltrate, although considerably diminished, was not entirely destroyed. In one instance the initial rotatory value of the ultrafiltrate before glycolysis proved to be levorotatory and in all instances ultrafiltrates

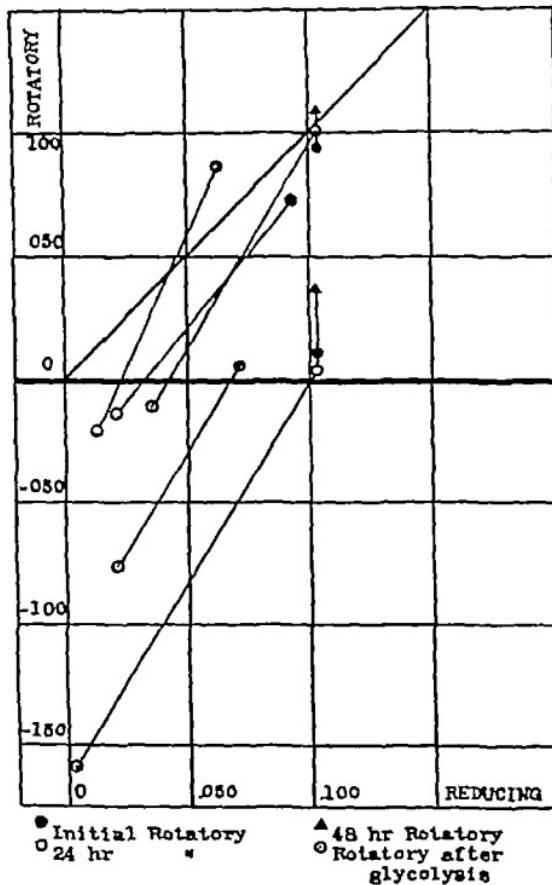


FIG. 3 REDUCING AND ROTATORY VALUES BEFORE AND AFTER GLYCOLYSIS

from the glycolyzed specimens on which the reducing values had been diminished to 40 mgm of sugar per 100 cc. or below, were levorotatory. The diminution in rotatory value in all instances proved to be either approximately equal to the diminution in the reducing values, if

calculated on the basis of the standard specific rotation of glucose, or considerably greater. That is, after the removal of glucose (R-P) was unchanged or increased, never diminished.

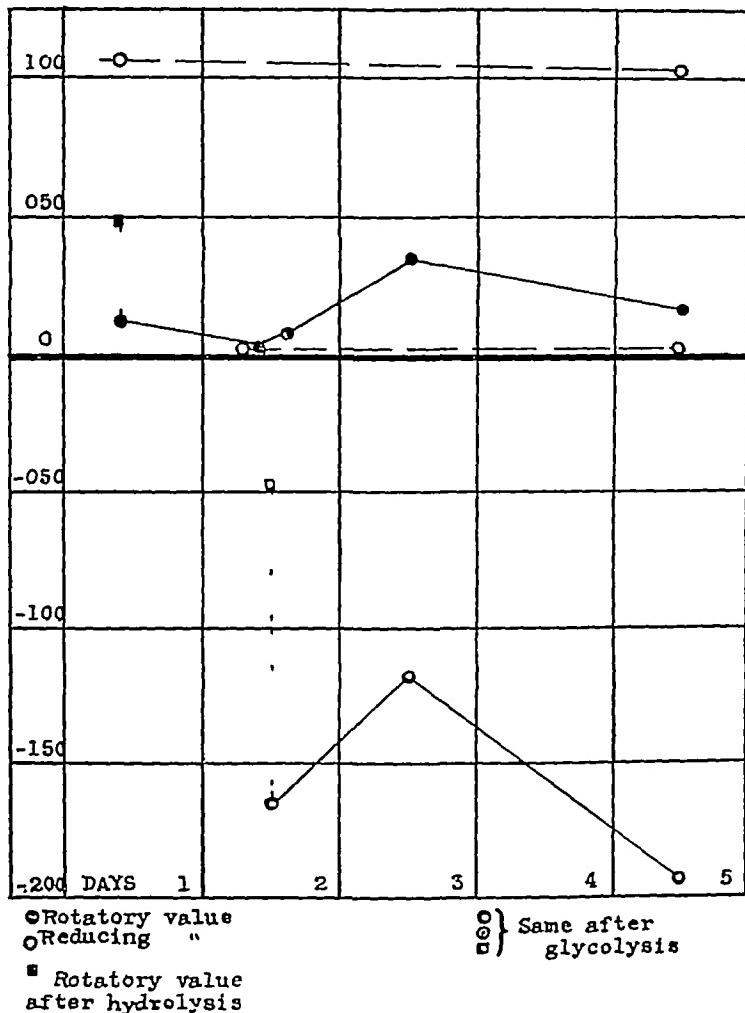


FIG 4 ROTATORY VALUES ON CONSECUTIVE DAYS BEFORE AND AFTER GLYCOLYSIS

It is evident, however, that our interpretation of the results from such an experiment should be guarded, as a number of variable factors complicate the problem. In the first place the relative instability of the rotatory values of ultrafiltrates from the unglycolyzed speci-

men, as shown by daily fluctuation, renders the accuracy of a comparison with the rotatory values of the glycolyzed specimen somewhat questionable. Furthermore, the results indicate that optically active substances other than glucose are increased or their optical activity is altered during the destruction of the sugar. Lepine (3) has emphasized the fact that if glycolysis of whole blood is allowed to proceed levorotatory substances are increased. The degree to which this variable may influence the results has not been determined, but it is evident that following the glycolytic action of whole blood one does not obtain rotatory values which are similar to the values which might be expected from the simple removal of glucose per se.

TABLE 5  
*Rotatory values on consecutive days before and after glycolysis*

	Reducing		Rotatory values			Reducing final gram per 100 cc
	Initial	Initial	24-hour	48-hour	96-hour	
	gram per 100 cc	gram per 100 cc	gram per 100 cc.	gram per 100 cc	gram per 100 cc	
Unglycolyzed specimen	0.106	+0.012	0.000	+0.035	+0.018	0.102
Glycolyzed specimen	0.000		-0.164	-0.118	-0.196	0.000

An interesting finding, however, which is graphically shown by a later experiment (fig. 4) was that after hydrolysis of both glycolyzed and unglycolyzed specimens, a comparison of the resulting (R-P) values showed them to be approximately equal. This finding suggests that more than one factor contributes to the difference between reducing and rotatory values, or (R-P) values, and that a certain fraction of this value is due the presence of levorotatory substances which are destroyed or rendered optically inactive on boiling with HCl. It suggests further that the increase in levorotation after glycolysis represents essentially an increase of this fraction of relatively unstable levorotatory substances.

Although the method of removing glucose by means of the glycolytic action of whole blood is open to criticism, it was felt that a study of the daily fluctuations of rotatory values after glycolysis might be of significance.

This experiment was performed on a sample of defibrinated ox

blood following the procedure utilized in the previous glycolysis experiments. The ultrafiltrates from both the original and the glycolyzed specimen were kept for several days and readings of each were obtained at intervals during this period. The results are shown in table 5 and figure 4.

The fluctuations exhibited by the rotatory values of the original unglycolyzed ultrafiltrate showed little change in the first twenty-four hours, followed by a moderate rise in the forty-eight-hour reading and a subsequent fall to the original value in ninety-six hours. In the glycolyzed specimen also the forty-eight-hour reading showed a sharp rise followed by an abrupt fall at the end of ninety-six hours. The experiment proved clearly that if sugar is removed from the blood by the glycolytic action of blood cells and the rotatory values of this sugar free ultrafiltrate is studied over a period of four days definite fluctuations in these values are recorded which show an approximate, although not an exact, parallelism to similar daily fluctuations of the rotatory readings from the original unglycolyzed specimen.

#### SUMMARY

In a series of comparative studies of the rotatory and reducing values of 18 ultrafiltrates from blood plasma of normal individuals and general hospital patients, exclusive of diabetics and nephritics, it was found that initially (i.e. twenty-four hours after collection of the sample) agreement of the rotatory and reducing values was recorded in only two instances. In the others rather wide irregular differences were noted, which have been designated in these experiments as (R-P) values and if expressed in terms of glucose these values were found to range between 0.010 and 0.130 gram per 100 cc. averaging about 0.040 gram per 100 cc. Occasionally the initial rotatory values proved to be levorotatory. In studying the rotatory values of the same ultrafiltrate over a period of consecutive days definite fluctuations were noted whereas the reducing values remained unchanged. These fluctuations of the rotatory values were distinctly irregular but generally showed a tendency to rise at some time during the first forty-eight hours after which time there was either a subsequent fall or little if any change. In two instances the rotatory values rose on standing.

to equal the reducing values. On hydrolyzing the ultrafiltrates it was found that the (R-P) values were often very appreciably diminished and in some instances became zero. In ultrafiltrates made after the blood sugar had been partially or wholly removed through the glycolytic action of whole blood the rotatory values became levorotatory, the difference between reducing and rotatory values being either equal to, or greater than that noted in an ultrafiltrate from the same blood before glycolysis. However, after hydrolysis of both glycolyzed and unglycolyzed specimens, a comparison of the (R-P) values showed them to be approximately equal.

Another interesting finding proved to be that, if the rotatory values of an ultrafiltrate from these glycolyzed specimens were studied over a period of consecutive days, pronounced fluctuations were observed which were similar to the fluctuations of the rotatory values occurring in the ultrafiltrate from an unglycolyzed sample of the same blood.

In general, therefore, the findings show that levorotatory substances (which may or may not have slight reducing power) are normally present in blood. It has been shown that this levorotation is decreased on hydrolysis and generally increased if the glycolytic action of whole blood has been allowed to proceed. There is further suggestive evidence which is not, however, conclusive, that the relative instability and daily fluctuations exhibited by these levorotatory substances on standing are responsible for the somewhat similar fluctuations of the rotatory values which have been noted so frequently in normal blood, an interpretation which has been given by many other investigators, notably Visccher (18) and Macleod (15).

In considering how far the evidence presented by these experiments is in accord with theories which suggest that part of the glucose present in blood is of lower specific rotation than  $\alpha, \beta$  glucose, it is clear that one cannot explain the entire (R-P) value on such a basis. The results furnish evidence of the presence of relatively unstable levorotatory substances which probably have little or no reducing power but no evidence of the presence of substances presenting the properties either of  $\gamma$ -glucose or the "new glucose" of Lundsgaard and Holbøll. They do not exclude the existence of such substances which might be present together with  $\alpha, \beta$  glucose and the levorotatory

substances noted above, but they suggest another explanation of the observed phenomena without postulating the presence of  $\gamma$ -glucose or "new glucose."

It is not within the scope of this paper to discuss the nature of the levorotatory substances which have been referred to above. Such substances might include conjugated glucuronic acids, amino and fatty acids, etc., but in all probability one of the chief levorotatory substances is conjugated glucuronic acid which has been demonstrated in the blood of normal human beings and animals by Mayer (2), Lepine (3), and Stepp (11). The presence of glucuronic acid in urine is well recognized as being largely responsible for the levorotatory values of normal urine, and these rotatory values have also been shown to exhibit definite fluctuations on standing (25).

In conclusion I wish to express my appreciation to Miss E F Herr for assistance in the analytical work and to Dr J H Austin for his helpful criticism during the course of the experiments.

#### CONCLUSIONS

The evidence brought forward by these experiments confirms the presence, often noted by others, of levorotatory substances in normal blood and suggests that changes in these optically active substances other than glucose, whatever their nature may be, are largely responsible for the fluctuations of the rotatory values observed under the conditions of these experiments in ultrafiltrates from plasma.

#### BIBLIOGRAPHY

- 1 Hiller, A., Linder, G C, and Van Slyke, D D *Jour Biol Chem*, 1925, lxiv, 625 The Reducing Substances of the Blood
- 2 Mayer, P *Zeit Physiol Chem*, 1901, xxxii, 518 Über eine bisher unbekannte reducirende Substanz des Blutes
- 3 Lepine, R, and Boulud *Compt rend des Sc de l'Acad d Sci*, 1901, cxxxii, 720 Physiologie Sur les Sucres du Sange et leur Glycolyse  
Lepine, R, and Boulud *Compt rend des Sc de l'Acad d Sci*, 1902, cxxxiv, 398 Chimie Animale Sur le Dosage des Sucres dans le Sang  
Lepine, R, and Boulud *Compt rend des Sc de l'Acad d Sci*, 1902, cxxxv, 139 Chimie Animale Sur l'Acide Glycuronique dans le Sang du Chien
- 4 Michaelis, L, and Rona, P *Biochem Zeit*, 1908, xiv, 476 Untersuchungen über den Blutzucker IV Die Methode der osmotischen Kompenstation

- 5 Rona, P., and Takahashi, D. Biochem Zeit., 1910, xxx, 99 Untersuchungen über den Blutzucker VIII Über den Zuckergehalt der Blutkörperchen
- 6 Oppler, B. Zeit Physiol. Chem., 1910, lxiv, 393 Zur Methodik der quantitativen Traubenzuckerbestimmung des Blutes.
- Oppler, B. Zeit Physiol. Chem., 1911, lxxv, 71 Die Bestimmung des Traubenzuckers in Harn und Blut
- 7 Lyttkens, H., and Sandgren, J. Biochem Zeit., 1911, xxx, 153 Über die Verteilung der reduzierenden Substanzen im Menschenblut
- 8 Griesbach, W., and Strassner, H. Zeit. Physiol. Chem., 1913, lxxxviii, 199 Zur Methodik der Blutzucker Bestimmung
- 9 Maase, C., and Tachau, H. Zeit. f. klin. med., 1915, lxxx, 1 Vergleichende Blutzuckerbestimmungen durch Polarisation und Reduktionsmethoden
- 10 Stepp, W. Zeit. Physiol. Chem., 1919, cvi, 29 Beiträge zur Kenntniss der reduzierenden Substanzen des Blutes.
- Stepp, W. Arch. f. exp. Path. u. Pharm., 1921, xc, 105 Kritisch analytische Betrachtungen und Untersuchungen zur Bestimmung des wahren Blutzuckers beim Gesunden und Kranken
- Stepp, W. Ergebn. Physiol., 1922, xx, 108 Über einige den Blutzucker betreffende Fragen im Lichte neueren Forschungsergebnisse.
- 11 Stepp, W. Zeit. Physiol. Chem., 1919, cvi, 264 Über das Vorkommen von Glukuronsäure im Menschlichen Blute.
- 12 Winter, L. B., and Smith, W. Jour. Physiol., 1923, lvii, 100 On the Nature of the Sugar in the Blood
- Forrest, W. D., Smith, W., and Winter, L. B. Jour. Physiol., 1923, lvii, 224 On the Change in the Nature of the Blood Sugar of Diabetics Caused by Insulin
- 13 Hewitt, J. A. Brit. Med. Jour., 1923, i, 590 Nature of Sugar in Blood
- 14 Eadie, G. S. Brit. Med. Jour., 1923, ii, 60 Behavior of Blood Sugar under Action of Insulin and Other Agents
- 15 Macleod, J. J. R. Physiol. Rev., 1924, iv, 21 Insulin
- 16 Van Creveld, S. Biochem. Jour., 1923, xvii, 860 Some experiments and Remarks on the Possible Transformation of d-Glucose in the Intestine and on the Nature of Blood Sugar
- 17 Denis, W., and Hume, H. V. Jour. Biol. Chem., 1924, ix, 603 On the Nature of Blood Sugar
- 18 Visscher, M. B. Amer. Jour. Physiol., 1926, lxxvi, 59 A Critical Study of the Evidence for the Presence of an Isomer of d-Glucose in Blood.
- 19 Lundsgaard, C., and Holbøll, S. A. Jour. Biol. Chem., 1925, lxxv, 323 and 343 Studies in Carbohydrate Metabolism. III and IV
- 20 Lundsgaard, C., and Holbøll, S. A. Jour. Biol. Chem., 1924, lxii, 453 Effect of Insulin and Muscle Tissue on Glucose in Vitro
- Lundsgaard, C., and Holbøll, S. A. Jour. Biol. Chem., 1925, lxxv, 305 Studies in Carbohydrate Metabolism II

21. Eggerth, A H Jour Biol Chem , 1921, xlvi, 203 The Preparation and Standardization of Collodion Membranes
- 22 Marshall, E K , and Vickers, J L Bull Johns Hopkins Hosp , 1923, xxxiv, 1 The Mechanism of the Elimination of Phenolsulphonephthalein by the Kidney A Proof of Secretion by the Convoluted Tubules
- 23 Folin, O , and Wu, H Jour Biol Chem , 1919, xxxviii, 81 A System of Blood Analysis  
Folin, O , and Wu, H Jour Biol Chem , 1920, xli, 367 A System of Blood Analysis Supplement 1
- 24 Host, H F , and Hatlehol, R Jour Biol Chem , 1920, xli, 347 Blood Sugar Concentration and Blood Sugar Methods
- 25 Paul, J R Jour Cln Invest , 1925, 1, 317 The Optical Activity of Glucose as Influenced by Normal and Diabetic Urine
- 26 Anderson, A B , and Carruthers, A Biochem Jour , 1926, xx, 556 Studies in Carbohydrate Metabolism I The Relation Between Optical Activity and Reducing Power of Normal Blood Filtrates, Interaction of Muscle Tissue, Insulin and Glucose

## INDEX TO VOLUME III

- Acid Base Equilibrium of Plasma in Health and Disease VIII Bicarbonate and Chloride in the Serum of Patients with Heart Failure John P Peters, Harold A Bulger and Anna J Eisenman, 497
- Acid Base Equilibrium of Plasma in Health and Disease IX High Serum Bicarbonate in Heart Failure Asphyctic Anoxemia John P Peters, Harold A Bulger and Anna J Eisenman, 511
- Ammonium Chlorid and Organic Mercury Compounds, A Study of the Action of Norman M Keith and Mary Whelan, 149
- Andrus, E Cowles, The Mechanism of the Action of the Hydrogen Ion upon the Cardiac Rhythm, 555
- Anoxemia, Asphyctic, Total Acid Base Equilibrium and Plasma in Health and Disease. IX. High Serum Bicarbonate in Heart Failure John P Peters, Harold A. Bulger and Anna J Eisenman, 511
- Aortic and Pulmonic Stenoses, Effect of Emilie Holman and Claude S Beck, 283
- Arterolar Sclerosis, The Association of Generalized, with High Blood Pressure and Cardiac Hypertrophy in Chronic Nephritis Arnold Branch and Geoffrey C Linder, 299
- Arthritis and Syphilis, An Experimental Study of the Synovial Fluid of Patients with Alan M Cheaney, Jarold E Kemp and Frederick H Baetjer, 131
- Auricular Fibrillation, the Effect of Rapid Irregular Rhythms as Seen in The Effect of Tachycardia on the Blood Flow in Dogs. Harold J Stewart, J Hamilton Crawford and A B Hastings, 435
- Austin, J H , Studies in Serum Electrolytes I Concentration of Electrolytes and Non-electrolytes in the Serum during Lobar Pneumonia, 37
- Baetjer, Frederick H , An Experimental Study of the Synovial Fluid of Patients with Arthritis and Syphilis, 131
- Barnett, G D , The Effect of Breathing Oxygen-enriched Air during Exercise upon Pulmonary Ventilation and upon the Lactic Acid Content of Blood and Urine, 317
- Beck, Claude S , The Physiological Response of the Circulatory System to Experimental Alterations III The Effect of Aortic and Pulmonic Stenoses, 283
- Bell J R , Studies on Red Cell Diameter I In Health and in Pernicious Anemia, 229
- Blake, Francis G , Studies in Scarlet Fever II The Relation of the Specific Toxemia of Scarlet Fever to the Course of the Disease, 397
- Blood Flow in Dogs, the Effect of Tachycardia on the, I The Effect of Rapid Irregular Rhythms as Seen in Auricular Fibrillation Harold J Stewart, J Hamilton Crawford and A B Hastings, 435
- Blood Flow in Dogs, the Effect of Tachycardia on the, II The Effect of Rapid Regular Rhythm Harold J Stewart and J Hamilton Crawford, 449
- Blood Flow, Variations in, with Changes in Position in Normal and Pathologic Subjects John S Lawrence, Lewis M Hurxthal and A. V Bock, 613
- Blood Hydration, The Plasma Proteins in Relation to III The Plasma Proteins in Malnutrition John P Peters, Maurice A. Wakeman and Anna J Eisenman, 491

- Blood Plasma, A Comparative Study of the Rotatory and Reducing Properties of Ultra-filtrates from John R Paul, 631
- Blood Plasma, The Effectiveness of Ultrafiltration for Quantitatively Determining the "Free Water" Content of, and for Estimating Physical-chemical Changes of the Plasma Proteins in Edema Margarete Meta Kunde, 577
- Blood Pressure, High, The Association of Generalized Arteriolar Sclerosis with, and Cardiac Hypertrophy in Chronic Nephritis Arnold Branch and Geoffrey C Linder, 299
- Blood Serum, Bicarbonate and Chloride in Patients with Heart Failure John P Peters, Harold A Bulger and Anna J Eisenman, 497
- Blood Serum, High, Bicarbonate in Heart Failure Asphyctic Anoxemia John P Peters, Harold A Bulger and Anna J Eisenman, 511
- Blood Serum, the Surface Tension of the, in Nephritis Louis Leiter, 267
- Bloodsugar Regulating Mechanism, Benign Glycosuria due to Disturbance in the Knud Faber, 203
- Bock, A V , Variations in Blood Flow with Changes in Position in Normal and Pathologic Subjects, 613
- Branch, Arnold, The Association of Generalized Arteriolar Sclerosis with High Blood Pressure and Cardiac Hypertrophy in Chronic Nephritis, 299
- Brown, George E , Calorimetric Studies of the Extremities III Clinical Data on Normal Subjects with Localized Vascular Disease, 369
- Bulger, Harold A , Total Acid-Base Equilibrium of Plasma in Health and Disease VIII Bicarbonate and Chloride in the Serum of Patients with Heart Failure, 497
- Bulger, Harold A , Total Acid-Base Equilibrium of Plasma in Health and Disease IX. High Serum Bicarbonate in Heart Failure Asphyctic Anoxemia, 511
- Calorimetric Studies of the Extremities I Theory and Practice of Methods Applicable to Such Investigations Charles Sheard, 327
- Calorimetric Studies of the Extremities II Experimental Apparatus and Procedures Roy Kegerreis, 357
- Calorimetric Studies of the Extremities III Clinical Data on Normal Subjects with Localized Vascular Disease George E Brown, 369
- Camac, J G , Studies in Serum Electrolytes I Concentration of Electrolytes and Non-electrolytes in the Serum during Lobar Pneumonia, 37
- Cardiac Areas of Dogs A Technique for Measuring X-ray Photographs of the Harold J Stewart, 475
- Cardiac Hypertrophy, The Association of Generalized Arteriolar Sclerosis with High Blood Pressure and, in Chronic Nephritis Arnold Branch and Geoffrey C Linder, 299
- Cardiac Out-put of the Dog, The Effects of Digitalis on the T R Harrison and Bernard W Leonard, 1
- Cardiac Rhythm, The Mechanism of the Action of the Hydrogen Ion upon the E Cowles Andrus and Edward P Carter, 555
- Carter, Edward P , The Mechanism of the Action of the Hydrogen Ion upon the Cardiac Rhythm, 555
- Chesney, Alan M , An Experimental Study of the Synovial Fluid of Patients with Arthritis and Syphilis, 131
- Chloride Concentration Test of de Weselow, Influence of Previous Salt Régime on Excretions of Chlorine, Sodium and Potassium during the Louis Leiter, 253
- Chronic Nephritis, The Association of Generalized Arteriolar Sclerosis with High Blood Pressure and Cardiac Hypertrophy in Arnold Branch and Geoffrey C Linder, 299
- Crawford, J Hamilton, The Effect of Regular and Irregular Tachycardias on the Size of the Heart, 483

- Crawford, J Hamilton, The Effect of Tachycardia on the Blood Flow in Dogs I The Effect of Rapid Irregular Rhythms as Seen in Auricular Fibrillation, 435
- Crawford, J Hamilton, The Effect of Tachycardia on the Blood Flow in Dogs II The Effect of Rapid Regular Rhythm, 449
- Davies, J A V, Studies in Scarlet Fever IV Post scarlatinal Immunity in Patients Treated with Antitoxin, 423
- Digitalis and Diuretics in Heart Failure with Regular Rhythm, with Especial Reference to the Importance of Etiologic Classifications of Heart Disease H M Marvin, 521
- Digitalis, The Effect of, on the Cardiac Out put of the Dog T R Harrison and Bernard W Leonard, 1
- Diuretics and Digitalis in Heart Failure with Regular Rhythm, with Especial Reference to the Importance of Etiologic Classifications of Heart Disease. H M Marvin, 521
- Edema II The Effectiveness of Ultra filtration for Quantitatively Determining the "Free Water" Content of Blood Plasma, and for Estimating Physical-chemical Changes of the Plasma Proteins in Edema Margarette Meta Kunde, 577
- Eisenman, Anna J, The Plasma Proteins in Relation to Blood Hydration III The Plasma Proteins in Malnutrition, 491
- Eisenman, Anna J, Total Acid Base Equilibrium of Plasma in Health and Disease. VIII Bicarbonate and Chloride in the Serum of Patients with Heart Failure, 497
- Eisenman, Anna J, Total Acid Base Equilibrium of Plasma in Health and Disease IX High Serum Bicarbonate in Heart Failure. Asphyctic Anoxemia, 511
- Electrocardiogram, Dependence of the Form of the, upon the Site of Mechanical Stimulation of the Human Ventricles B S Oppenheimer and Harold J Stewart, 593
- Electrocardiograms of Low Voltage, The Significance of Howard Sprague and Paul D White, 109
- Epidemic Prevalence in the Light of Experimental Findings L T Webster, 465
- Epinephrin Reaction in Obesity Clement I Krantz and James H Means, 565
- Faber, Knud Benign Glycosuria Due to Disturbances in the Bloodsugar Regulating Mechanism, 203
- Free Water Content, The Effectiveness of Ultrafiltration for Quantitatively Determining the, of Blood Plasma and for Estimating Physical-chemical Changes of the Plasma Proteins in Edema Edema II Margarette Meta Kunde, 577
- Glycosuria, Benign, Due to Disturbances in the Bloodsugar Regulating Mechanism Knud Faber, 203
- Harrison, T R., The Effects of Digitalis on the Cardiac Out-put of the Dog, 1
- Hastings, A B, The Effect of Tachycardia on the Blood Flow in Dogs I The Effect of Rapid Irregular Rhythms as Seen in Auricular Fibrillation, 435
- Heart, the Effect of Regular and Irregular Tachycardias on the Size of the Harold J Stewart and J Hamilton Crawford, 483
- Heart Disease, the Effect of Increased Heart Rate Due to the Injection of Atropine on the Oxygen Saturation of the Arterial and Venous Blood of Patients with Harold J Stewart, 241
- Heart Failure, Bicarbonate and Chloride in the Serum of Patients with John P Peters, Harold A Bulger and Anna J Eisenman 497

- Heart Failure, Digitalis and Diuretics in, with Especial Reference to the Importance of Etiologic Classifications of Heart Disease H M Marvin, 521
- Heart Failure, High Serum Bicarbonate in John P Peters, Harold A Bulger and Anna J Eisenman, 511
- Heart Rate, The Effect of Increased, due to the Injection of Atropine, on the Oxygen Saturation of the Arterial and Venous Blood of Patients with Heart Disease Harold J Stewart, 241
- Hewlett, A W , The Effect of Breathing Oxygen-enriched Air during Exercise upon Pulmonary Ventilation and upon the Lactic Acid Content of Blood and Urine, 317
- Holman, Emile, The Physiological Response of the Circulatory System to Experimental Alterations III The Effect of Aortic and Pulmonic Stenoses, 283
- Hurthal, Lewis M , Variations in Blood Flow with Changes in Position in Normal and Pathologic Subjects, 613
- Hydrogen Ion, The Mechanism of the Action of, upon the Cardiac Rhythm E Cowles Andrus and Edward P Carter, 555
- Kegerreis, Roy, Calorimetric Studies of the Extremities II Experimental Apparatus and Procedures, 357
- Keith, Norman M , A Study of the Action of Ammonium Chlorid and Organic Mercury Compounds, 149
- Kemp, Jarold E , An Experimental Study of the Synovial Fluid of Patients with Arthritis and Syphilis, 131
- Kidney Function in Pneumonia John F McIntosh and Hobart A Reimann, 123
- Krantz, Clement I , Epinephrin Reaction in Obesity, 565
- Kunde, Margarete Meta, Edema II The Effectiveness of Ultrafiltration for Quantitatively Determining the "Free Water" Content of Blood Plasma, and for Estimating Physical-chemical Changes of the Plasma Proteins in Edema, 577
- Lactic Acid Content of Blood and Urine, the Effect of Breathing Oxygen-enriched Air during Exercise upon Pulmonary Ventilation and upon the A W Hewlett, G D Barnett and J K Lewis, 317
- Lawrence, John S , Variations in Blood Flow with Changes in Position in Normal and Pathologic Subjects, 613
- Leiter, Louis, Influence of Previous Salt Régime on Excretions of Chlorine, Sodium, and Potassium during the Chloride Concentration Test of de Wesselow, 253
- Leiter, Louis, The Surface Tension of the Blood Serum in Nephritis, 267
- Leonard, Bernard W , The Effects of Digitalis on the Cardiac Out-put of the Dog, 1
- Lewis, J K , The Effect of Breathing Oxygen-enriched Air during Exercise upon Pulmonary Ventilation and upon the Lactic Acid Content of Blood and Urine, 317
- Linder, Geoffry C , The Association of Generalized Arteriolar Sclerosis with High Blood Pressure and Cardiac Hypertrophy in Chronic Nephritis, 299
- Lobar, Pneumonia, Concentration of Electrolytes and Non-electrolytes in the Serum during F William Sunderman, J H Austin and J G Camac, 37
- Malnutrition, The Plasma Proteins in Relation to Blood Hydration John P Peters, Maurice A Wakeman and Anna J Eisenman, 491
- Marvin, H M , Digitalis and Diuretics in Heart Failure with Regular Rhythm, with Especial Reference to the Importance of Etiologic Classifications of Heart Disease, 521
- Means, James H , Epinephrin Reaction in Obesity, 565

- Means, J H., Studies on Red Cell Diameter I In Health and in Pernicious Anemia, 229
- Medearis, Donald N., Studies on Red Blood Cell Diameter II In Pernicious Anemia, Before and During Marked Remission, and in Myelogenous Leukemia, 541
- Mercury Compounds, Organic, A Study of the Action of Ammonium Chloride and Norman M Keith and Mary Whelan, 149
- Minot, George R., Studies on Red Blood Cell Diameter II In Pernicious Anemia, Before and During Marked Remission and in Myelogenous Leukemia, 541
- Myelogenous Leukemia, Studies on Red Cell Diameter in Donald M Medearis and George R Minot, 541
- McIntosh, John F., Kidney Function in Pneumonia, 123
- Nephritis, The Surface Tension of the Blood Serum in Louis Leiter, 267
- Nicholls Edith, Studies in Scarlet Fever III Infections with Streptococcus Scarletinae in Persons with Scarletinal Antitoxin Immunity, 411
- Obesity, Epinephrin Reaction in Clement L Krantz and James H Means, 565
- Oppenheimer, B S, Dependence of the Form of the Electrocardiogram upon the Site of Mechanical Stimulation of the Human Ventricles, 593
- Oxygen-enriched Air, the Effect of, during Exercise upon Pulmonary Ventilation and upon the Lactic Acid Content of Blood and Urine. A W Hewlett, G D Barnett and J K Lewis, 317
- Paul, John R., A Comparative Study of the Rotatory and Reducing Properties of Ultrafiltrates from Blood Plasma 631
- Pernicious Anemia, Studies on Red Blood Cell Diameter in Donald N Medearis and George R. Minot, 541
- Pernicious Anemia, Studies on Red Cell Diameter I In Health and in J R Bell, F K. Thomas and J H Means, 229
- Peters, John P., The Plasma Proteins in Relation to Blood Hydration III. The Plasma Proteins in Malnutrition, 491
- Peters, John P., Total Acid Base Equilibrium of Plasma in Health and Disease. VIII Bicarbonate and Chloride in the Serum of Patients with Heart Failure, 497
- Peters, John P., Total Acid Base Equilibrium of Plasma in Health and Disease. IX. High Serum Bicarbonate in Heart Failure Asphyctic Anoxemia, 511
- Plasma Proteins, The, in Relation to Blood Hydration III The Plasma Proteins in Malnutrition John P Peters, Maurice A. Wakeman, and Anna J Eisenman, 491
- Pneumonia, Kidney Function in, John F McIntosh and Hobart A Reimann, 123
- Pulmonic Stenoses, The Effect of Aortic and Emile Holman and Claude S Beck, 283
- Red Blood Cell Diameter, Studies on, II In Pernicious Anemia, before and during Marked Remission, and in Myelogenous Leukemia Donald M Medearis and George R. Minot, 541
- Red Cell Diameter, Studies on, I In Health and in Pernicious Anemia J R Bell, F K Thomas and J H Means, 229
- Reimann, Hobart A., Kidney Function in Pneumonia, 123
- Rotatory and Reducing Properties, A Comparative Study of the, of Ultrafiltrates from Blood Plasma John R Paul, 631

